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Synthesis and Preliminary Biological Evaluation of Furo- and Oxazolopyrimidine Analogues

Thesis submitted in fulfilment of the requirements for the degree of Doctor (PhD) in Applied Biological Sciences: Chemistry and Bioprocess Technology

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Woord vooraf

Met het schrijven van dit voorwoord komt er een einde aan mijn avontuur bij de Universiteit Gent, en kan ik terugkijken op een mooi hoofdstuk in mijn leven. In dit voorwoord zal ik het verloop van mijn traject aan de universiteit wat toelichten, en de mensen bedanken die hun bijdrage hebben geleverd aan deze thesis.

Het begon allemaal met de keuze om na mijn middelbare studies aan een opleiding tot bio-ingenieur te beginnen aan de Universiteit Gent. De grote hervorming van de bachelor/master-structuur was toen net achter de rug, en de opleiding ging van start met een algemene basis. Daarna kon ik mijn eigen richting bepalen met de keuze van een afstudeerrichting en de samenstelling van keuzevakken. Aan de richting die ik wou uitgaan binnen de opleiding tot bio-ingenieur heb ik eigenlijk nooit getwijfeld. Ik heb altijd een passie gehad voor chemie, en de Master in 'Chemie en Bioprocestechnologie' klonk dan ook als muziek in mijn oren. In het laatste jaar begon ik aan een Masterthesis binnen de vakgroep Duurzame Organische Chemie en Technologie onder het promotorschap van professor Stevens. Tijdens mijn thesis deed ik onderzoek naar de synthese van analogen van het natuurproduct zingeron met potentiële antikankereigenschappen. Ik kijk nog steeds met fierheid terug op mijn Masterthesis. Na mijn Masteropleiding kon ik instappen in een medicinaal IWT-SBO doctoraatsproject dat iets meer dan een jaar eerder van start was gegaan. Een doctoraatsstudent had na een jaar de overstap gemaakt naar een job in de farmaceutische industrie, en ik kon zijn positie overnemen. Dit betekende meteen ook dat ik minder dan drie jaar financiering had. Extra financiële middelen werden uiteindelijk bekomen na het schrijven en succesvol behalen van een BOF-project, waardoor ik uiteindelijk 3 jaar en 10 maanden officieel betaald werd om aan mijn doctoraat te werken. Tijdens deze periode heb ik mij met allerlei projecten beziggehouden. Zo waren er syntheseprojecten voor Galapagos, UZ Gent en VIB. Steeds nam ik enthousiast deze opdrachten aan, en allen resulteerden uiteindelijk in succes. Mede daardoor vond ik na iets minder dan vier jaar dat de resultaten van mijn eigen doctoraatsproject nog niet volstonden om te doctoreren, alhoewel technisch gezien aan de criteria voldaan werd. De mogelijkheid die zich toen aanbood was om als wetenschappelijk medewerker fulltime op onderzoeksprojecten te werken, en daar bovenop het doctoraatswerk af te ronden. Dit bleek uiteindelijk niet zo evident. Ik wou namelijk van deze onderzoeksprojecten ook een succes maken, en dit vereiste een grote inzet. Wat volgde waren onderzoeksprojecten voor Cargill, Creachem en een consortium van bedrijven in het ATOM-2 project, hetgeen resulteerde in een verbreding van mijn chemische expertise, en een aantal mooie onderzoeksrapporten. Uiteindelijk slaagde ik er ook in om mijn doctoraatsthesis af te ronden, na het opofferen van veel vrije tijd en vakantiedagen. Met het schrijven van deze tekst is het einde van mijn doctoraatsproject eindelijk nabij.

Tijdens mijn periode aan de universiteit heb ik heel veel verschillende mensen leren kennen. Zowel op het werk als naast het werk heb ik met deze mensen fijne momenten beleefd, maar het zou mij veel te ver leiden om al deze anekdotes hier neer te pennen.

Een doctoraat is niet enkel een persoonlijk project, het is ook teamwerk. Zonder de hulp van andere mensen zou het absoluut onmogelijk geweest zijn om dit te verwezenlijken. Eerst en vooral wil ik

professor Stevens bedanken, om mij steeds kansen te bieden en voor het vertrouwen dat u mij hebt geschonken. Ik heb steeds veel vrijheid gekregen om als wetenschapper mijn eigen weg te zoeken in alle projecten, waarvoor dank. Daarnaast stond uw deur steeds open om met vragen of problemen langs te komen. Ten tweede wil ik Bart bedanken. Telkens als ik raad of hulp nodig had, stond je klaar om te helpen. Je hebt me geholpen met mijn Masterthesis, met het BOF-project, bij het schrijven en publiceren van wetenschappelijke artikels en met zovele andere zaken… steeds kon ik op jou rekenen. Bedankt!

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De mensen die mijn moleculen hebben onderzocht op biologische activiteit hebben ook een grote bijdrage geleverd aan dit werk. Bij deze wil ik Prof. Naesens, Prof. Coenye en Prof. Müller bedanken voor al hun biologische resultaten.

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Laurens De Coen

Mei 2019

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List of abbreviations

2-AG	2-arachidonoylglycerol
AAA	ATPases associated with diverse cellular activities
ACC	acetyl coenzyme A
ADP	adenosine diphosphate
AEA	*N*-arachidonoylethanolamide
AK	adenosine kinase
AKI	acute kidney injury
AMP	adenosine monophosphate
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
ATBR	1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose
atm.	atmosphere
ATP	adenosine triphosphate
ATPase	adenylpyrophosphatase
ATR	attenuated total reflectance
BCCM	Belgian Co-ordinated Collections of Micro-organisms
BH_4	tetrahydrobiopterin
Boc	*tert*-butoxycarbonyl
BOP	(benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate
BRD	bromodomain 4
BUB	budding uninhibited by benzimidazoles
CAVD	calcific aortic valve disease
CDI	1,1'-carbonyldiimidazole
CLogP	calculated log P
CNS	central nervous system
COPD	chronic obstructive pulmonary disease
CPPD	calcium pyrophosphate deposition disease
dba	dibenzylideneacetone
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DCC	*N*,*N*'-dicyclohexylcarbodiimide
DIPEA	*N*,*N*-diisopropylethylamine
DMAP	4-(dimethylamino)pyridine
DMF	*N*,*N*-dimethylformamide
DMEDA	*N*,*N*'-dimethylethylenediamine
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DPPA	diphenylphosphoryl azide
EC50	half maximal effective concentration

EDCI	1-ethyl-3-(3-dimethylaminopropyl)carbodiimide
EDG	endothelial differentiation gene receptor
EGFR	epidermal growth factor receptor
EPS	extracellular polymeric substances
ESI	electrospray ionization
FAAH	fatty acid amide hydrolase
FBDD	fragment-based drug discovery
FDA	United States Food and Drug Administration
GDIS	glucose-dependent insulin secretion
GPCR	G protein-coupled receptor
GVHD	graft versus host disease
HALE	healthy life expectancy
HATU	1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxide hexafluorophosphate
HCV	hepatitis C virus
HGPRT	hypoxanthine-guanine phosphoribosyl transferase
HIV	human immunodeficiency virus
HMPT	hexamethylphosphoric triamide
HOAt	1-hydroxy-7-azabenzotriazole
HOBt	1-hydroxybenzotriazole
HRMS	high resolution mass spectrometry
HTS	high-throughput screening
HUVEC	human umbilical vein endothelial cell
IC_{50}	half maximal inhibitory concentration
IR	infrared spectroscopy
JAK	janus kinase
KA	kainic acid
Ki	inhibitory constant
LC-MS	liquid chromatography – mass spectrometry
*m*CPBA	*meta*-chloroperoxybenzoic acid
MGL	monoglyceride lipase
MIC	minimum inhibitory concentration
MLR	mixed lymphocyte reaction
Mol.	molecular
Mp	melting point
MS	mass spectrometry
mTOR	mammalian target of rapamycin
MTS	3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium
µW	microwave

NAE	NEDD8-activating enzyme
NMDA	*N*-methyl-D-aspartate
NME	new molecular entity
NMP	*N*-methyl-2-pyrrolidone
NMR	nuclear magnetic resonance
N,*O*-BSA	*N*,*O*-bis(trimethylsilyl)acetamide
NPP1	ectonucleotide pyrophosphatase/phosphodiesterase-1
Nu	nucleophile
o.n.	overnight
PDE	phosphodiesterase
PET	positron emission tomography
PK	plasma kallikrein
PKB	protein kinase B
PMB	*para*-methoxybenzyl
PPA	polyphosphoric acid
PSA	polar surface area
Rac-BINAP	racemic 2,2’-bis(diphenylphosphino)-1,1’-binaphthyl
RBC	red blood cell
RM	reaction mixture
ROCK	rho-associated protein kinase
RSV	respiratory syncytial virus
r.t.	room temperature
RTA	ricin toxin A chain
sat.	saturated
SEM	2-(trimethylsilyl)ethoxymethyl
SPR	sepiapterin reductase
Stx	shiga toxin
TBAI	tetrabutylammonium iodide
TFA	trifluoroacetic acid
TGF-β	transforming growth factor-beta
THF	tetrahydrofuran
TLC	thin layer chromatography
TMSOTf	trimethylsilyl trifluoromethanesulfonate
Ts	*para*-toluenesulfonyl
TRPV	transient receptor potential protein vanilloid receptor
UDA	*Urtica dioica* agglutinin
VEGFR	vascular endothelial growth factor receptor
WHO	World Health Organization
X-phos	2-dicyclohexylphosphino-2’,4’,6’-triisopropylbiphenyl

Introduction and goals

Data provided by the World Health Organization shows that the life expectancy is still increasing worldwide. For example, the life expectancy of a female at age 60 in Europe, has increased from 81.6 years in 2000 to 84.1 years in 2016. Also the healthy life expectancy (HALE) shows a steady increase worldwide over the past decade. Different studies support the hypothesis that biomedical research and innovation is responsible for a substantial portion of recent gains in health and life expectancy. Innovation in pharmaceuticals not only has an effect on longevity but also on the quality of life, for example the ability of people to engage in daily activities.[1]

Despite the fact that many pharmaceuticals have been developed already to treat a broad spectrum of medical conditions, the long list of currently incurable diseases (*e.g.* Alzheimer's disease, HIV, multiple sclerosis, Parkinson's disease, certain types of cancer,…) and the development of resistance by pathogens against existing medicines illustrate the need for new medicines. However, the pharmaceutical industry is challenged by increasing costs of research and development and a simultaneous stagnating number of new molecular entities (NMEs).[2] Data supplied by the US Food and Drug Administration (FDA) show that approximately between 20 and 40 NMEs were approved by the FDA each year during the past decade. Approximately 70% of those NMEs were small molecules, while the other 30% were biologics, and this value remained relatively constant. The stagnating number of NMEs is not caused by the biology as many new drug targets became available the last decade, for example by decoding of the human genome.[3] Medicinal chemistry proved to be the bottleneck in the search for new drugs.[4,5] Indeed, the challenge is to find drug-like molecules which not only interact with the most druggable targets, but also possess excellent pharmacokinetic and low toxicological properties.

A new drug discovery project which aims to find biologically active compounds can follow different approaches. For example, hits can be obtained by *in silico* screening methods. Very often however, drug discovery projects use high-throughput screening (HTS) of large commercially available compound libraries against the drug target. Although the combinatorial chemistry technique has contributed enormous amounts of new substances to these compound libraries, the number of identified hits did not increase proportionally.[6] This phenomenon was mainly attributed to the poor drug-like properties of the compound libraries and the poor chemical diversity.[4,5] A structural diversity analysis of 24 million organic compounds revealed that a small percentage of frameworks occurred in a large percentage of compounds and that half of the compounds could be described by only 143 framework shapes.[7] Similarly, an analysis of approximately 5000 drugs showed that only 32 frameworks described the shapes of 50% of the compounds.[8] Minimization of synthesis costs is one explanation for the poor diversity of compound libraries. Decorating an existing scaffold by replacing peripheral functional groups is much less complicated than manipulating the molecular framework.

In order to address the problem of poor chemical diversity, pharmaceutical researchers have been shifting their hit finding strategy from HTS to fragment-based drug discovery (FBDD).[9,10] One of the advantages of FBDD is that even a small collection of fragments (a few thousands) covers a much larger proportion of all the possible compounds that could exist, termed ‘chemical space’, than large compound libraries (millions) for HTS. For example, the estimated number of potential fragments with up to twelve non-hydrogen atoms (fragment-like chemical space) is 10^7, while for potential drug-like molecules with up to thirty non-hydrogen atoms, this number exceeds 10^{60} (drug-like chemical space).[11] Therefore, screening a proportion of the fragment-like chemical space in FBDD is much more feasible than screening a proportion of the drug-like chemical space in HTS.

Medicinal chemists have also been using compound libraries consisting of molecules based on privileged structures to increase hit rates in screening assays. Privileged structures are a class of molecules which can bind to multiple, unrelated targets with high affinity, thus providing the medicinal chemist with the possibility to discover biologically active compounds across a broad range of therapeutic areas by screening a compound library based upon one core scaffold.[12,13] These structures are typically rigid, heterocyclic multiring molecules which can hold their substituent patterns in a well-defined three-dimensional space, which allows for high-affinity binding interactions between the target and either or both of the scaffold and the substituents. Well-known examples of privileged scaffolds are the heterobicyclic indoles, coumarines, quinolines and benzimidazoles and the monocyclic isoxazolidines.[14–16]

The goal of this research is the design and synthesis of compound libraries, based on novel or highly underexplored scaffolds, thereby covering largely unexplored chemical space. These scaffolds should at the same time also possess adequate physicochemical properties. Thus, the most important issues are novelty and drug-likeness of the scaffolds. This approach will provide new molecules which will be useful for (fragment-based) screening across a broad range of therapeutic targets.

The scaffolds were selected from the class of heterobicyclic compounds, since these are known to act as privileged structures. Also, the molecular weight of these scaffolds is low enough to provide the possibility to improve the selectivity and affinity of hit compounds by decoration of the scaffold with suitable substituents (which will increase the molecular weight) without losing their drug-like character. The concept of drug-likeness was introduced by Lipinski after an analysis of small-molecule drugs showed that orally administered drugs are very frequently residing in areas of chemical space which are defined by a limited range of molecular properties.[17] Several descriptors and rules-of-thumb have been introduced to address the drug-likeness of a compound. A well-known example is Lipinski’s Rule of Five, which states that historically, 90% of orally absorbed drugs had no more than five hydrogen-bond donors, no more than ten hydrogen-bond acceptors, molecular masses of less than 500 Da and log P values not greater than five.[18,19] Besides oral absorption, discrimination between drugs and non-drugs can also be based on other characteristics such as aqueous solubility and permeability, which can both be assessed by a range of molecular properties and descriptors.[8,20–22] Examples of such molecular properties are the molecular weight, the calculated log P (CLogP), the polar surface area (PSA), the number of rotatable bonds and the number of hydrogen bonding groups.[23] For the construction of fragment libraries, the Rule of Three can be a useful guideline, which states that both the number of hydrogen-bond donors and the number of hydrogen-bond acceptors should not be higher than three, the molecular weight should be lower than 300 Da and the fragment-based predicted log P (cLogP) should not be higher than three.[24] These guidelines will be followed whenever it is appropriate.

A literature search was performed on a broad range of heterobicyclic scaffolds. Those scaffolds without any hits in SciFinder were not withheld, as they were considered to be synthetically intractable, or at least very hard to make. Only structures which have already been reported to a very limited extent were considered as underexplored, yet synthetically feasible scaffolds. Twenty-four scaffolds were selected, of which two will be covered in this PhD thesis: the furo[3,4-*d*]pyrimidine scaffold **1** and the oxazolo[4,5-*d*]pyrimidine scaffold **2** (Figure 1). Both scaffolds are composed of a pyrimidine ring fused to a 5-membered heteroaromatic ring and are related to pyrrolopyrimidine, which is a well-known privileged scaffold.[25] Examples of marketed drugs that contain the pyrrolopyrimidine scaffold are Forodesine **4** for the treatment of relapsed/refractory peripheral T-cell lymphoma and Pemetrexed **5** for the treatment of pleural mesothelioma and non-small cell lung cancer (Figure 2). A straightforward synthetic route towards these scaffolds needs to be developed which allows for decoration of the structure with a variety of substituents, so that a library of drug-like compounds can be designed and synthesized.

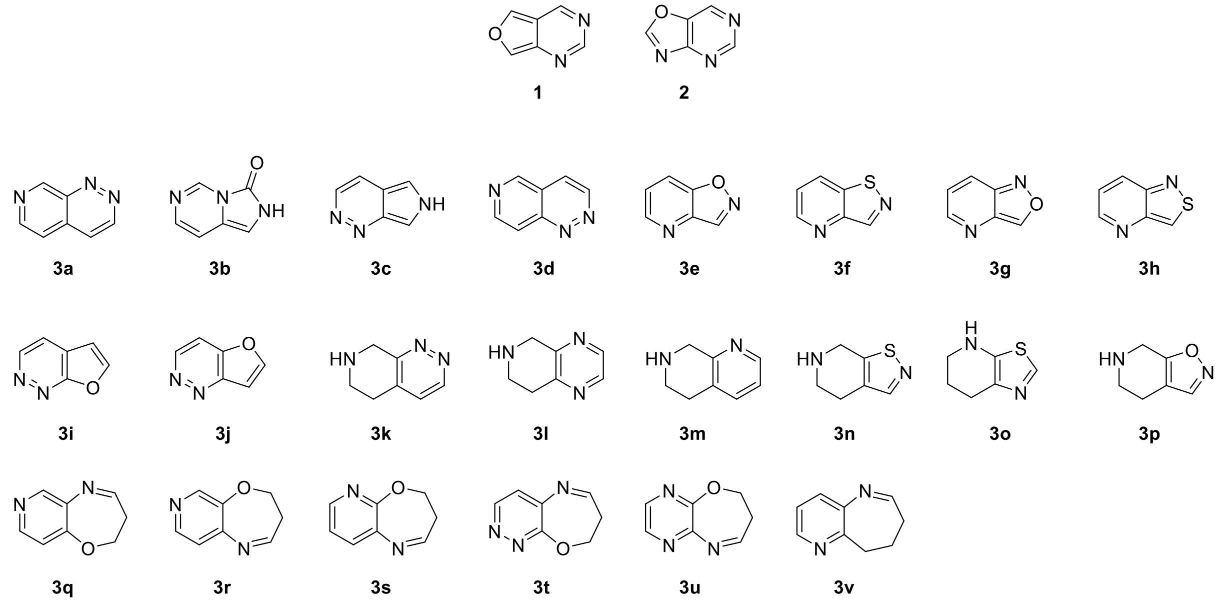
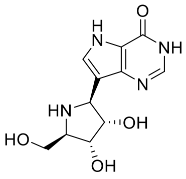
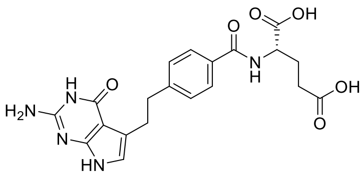


*Figure 1. The furo[3,4-d]pyrimidine (**1**) and oxazolo[4,5-d]pyrimidine (**2**) scaffolds which are covered in this PhD thesis, and the other bicyclic scaffolds **3a-v** which were selected for the medicinal project*



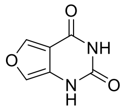
4, Forodesine



5, Pemetrexed

Figure 2. Marketed drugs containing the pyrrolopyrimidine privileged scaffold

Furthermore, modern medicinal chemistry is slowly abandoning the area of 'flat' aromatic molecules as an approach to improving clinical success. Lovering and coworkers demonstrated that the saturation of compounds correlates to their aqueous solubility, which is an important physicochemical parameter when it comes to success in drug discovery.[26] Therefore, the underexplored analogues furo[3,4-*d*]pyrimidine-2,4-dione **6**, oxazolo[4,5-*d*]pyrimidine-5,7-dione **7**, 5,7-dihydrofuro[3,4-*d*]pyrimidine-2,4-dione **8** and tetrahydrofuro[3,4-*d*]pyrimidine-2,4-dione **9**, characterized by a varying degree of non-flatness, are also considered to be interesting scaffolds for medicinal chemistry (Figure 3). In addition, the oxazolo[5,4-*d*]pyrimidine-5,7-dione scaffold **10** (bearing a hydrogen atom on *C*-2) is virtually unexplored as well.



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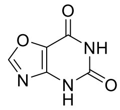

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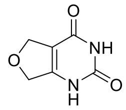

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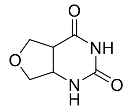

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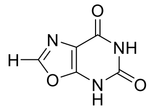
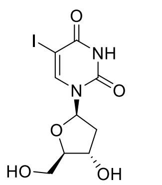


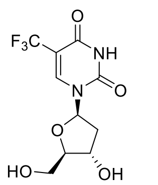
10

Figure 3. Unexplored/underexplored analogues of the intended furo[3,4-d]pyrimidine and oxazolo[4,5-d]pyrimidine scaffold with a varying degree of non-flatness

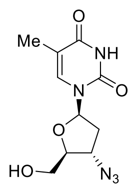
All of the molecules which are depicted in Figure 3 can be considered as analogues of uracil and thymine. Nucleoside analogues are a very important class of antiviral compounds, and examples of antiviral uridine/thymidine analogues are Idoxuridine **11**, Trifluridine **12** and Zidovudine **13** (Figure 4). The scaffolds **6-10** will be used to synthesize a small library of nucleoside analogues, which can be submitted to a broad antiviral screening.



11, Idoxuridine



12, Trifluridine



13, Zidovudine

Figure 4. Antiviral uridine/thymidine analogues Idoxuridine, Trifluridine and Zidovudine

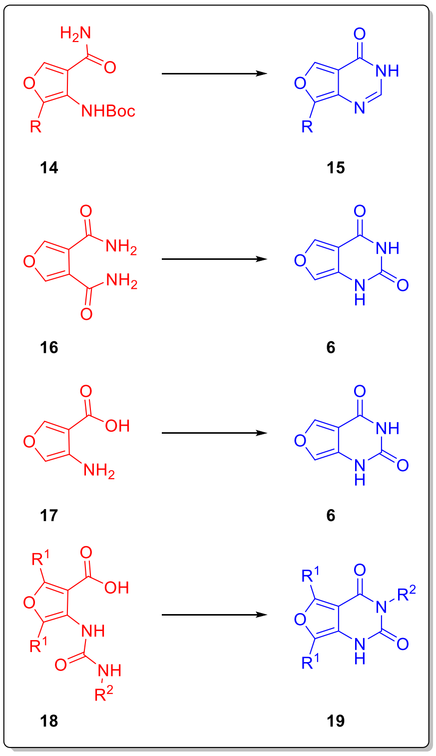
Literature overview

In this literature study, the synthesis of furo- and 5,7-dihydrofuro[3,4-*d*]pyrimidines, oxazolo[4,5-*d*]pyrimidines and oxazolo[5,4-*d*]pyrimidines will be reviewed. Focus will lay on the construction of the scaffold, rather than on further modification. Nevertheless, selected examples will illustrate some of the most common modifications of these scaffolds. The synthetic part is complemented by an overview of the biological activity of these structures. The synthesis of these bioactive compounds will not be described in detail, but the applied synthetic methodology for the construction of the scaffold is always included in the overview of synthetic methodologies. This review covers literature and patents from 1967 until 2018. The chapters on oxazolopyrimidines have been published in the European Journal of Organic Chemistry.[27]

1 Synthesis and biological activity of furo[3,4-*d*]pyrimidines

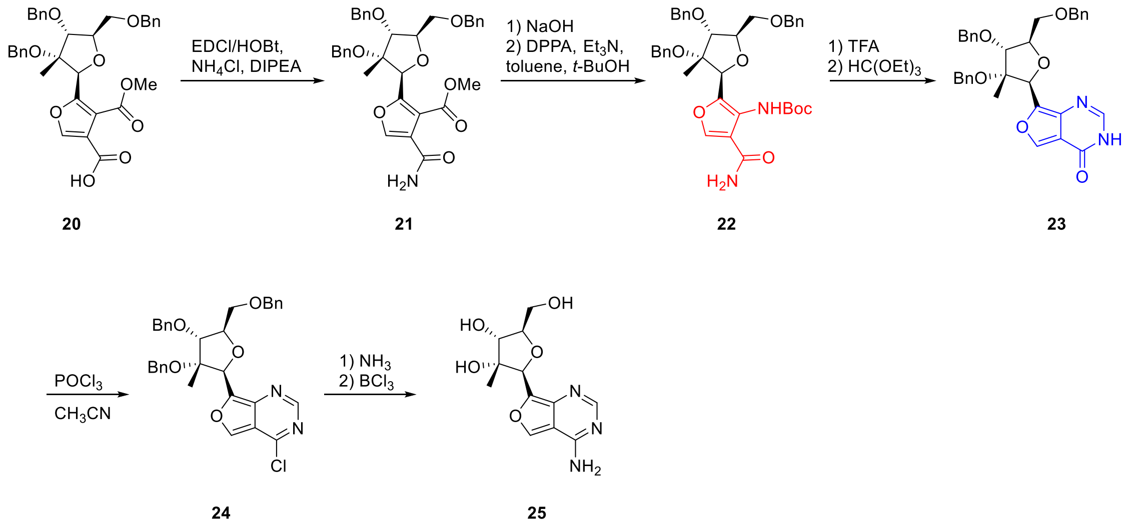
1.1 Synthesis of furo[3,4-*d*]pyrimidines

Furo[3,4-*d*]pyrimidines have been described only very scarcely in the literature. Four synthetic pathways have been exploited, all starting from a 3,4-difunctionalized furan ring. The scaffold was then constructed by pyrimidine annulation. A generic overview is given in Scheme 1.



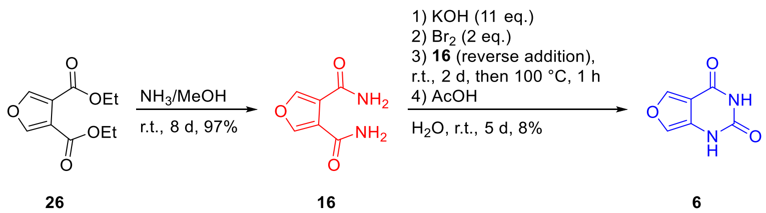
Scheme 1. Furo[3,4-d]pyrimidines by annulation of pyrimidine rings

Babu and coworkers reported on the synthesis of nucleoside analogue **25** (Scheme 2). Only the last part of the synthetic pathway is described here, starting from the highly functionalized furan carboxylic acid **20**. This carboxylic acid was transformed into the corresponding amide by a carbodiimide-mediated coupling reaction. Then, the ester was hydrolyzed and the resulting carboxylic acid was converted to a Boc-protected amine through a Curtius rearrangement. Deprotection of the amine and condensation with triethyl orthoformate afforded the furo[3,4-*d*]pyrimidinone **23**. Chlorination with phosphorus oxychloride, subsequent substitution with ammonia and deprotection of the ribose moiety afforded the desired nucleoside analogue **25**. Yields and detailed reaction conditions were not mentioned in the originating patent.[28]



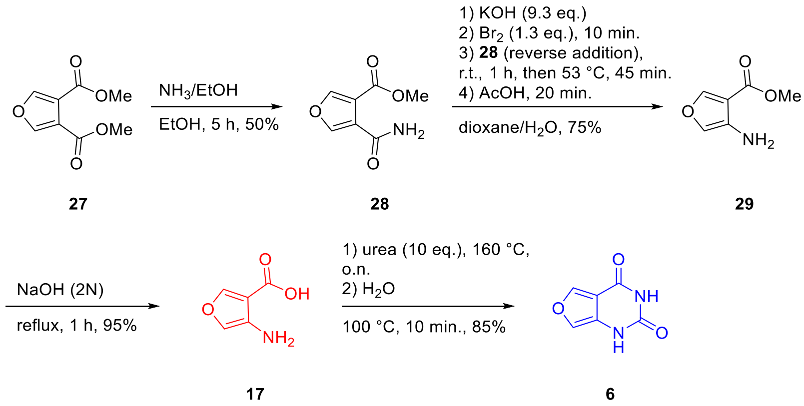
*Scheme 2. The synthesis of nucleoside analogue **25***

Jones developed a two-step pathway towards furo[3,4-*d*]pyrimidine-2,4-dione **6** starting from diethyl ester **26** (Scheme 3). Aminolysis of the ester delivered the diamide **16** in excellent yield. In contrast, the subsequent Hofmann rearrangement gave the furopyrimidinone **6** only in a very low yield.[29]



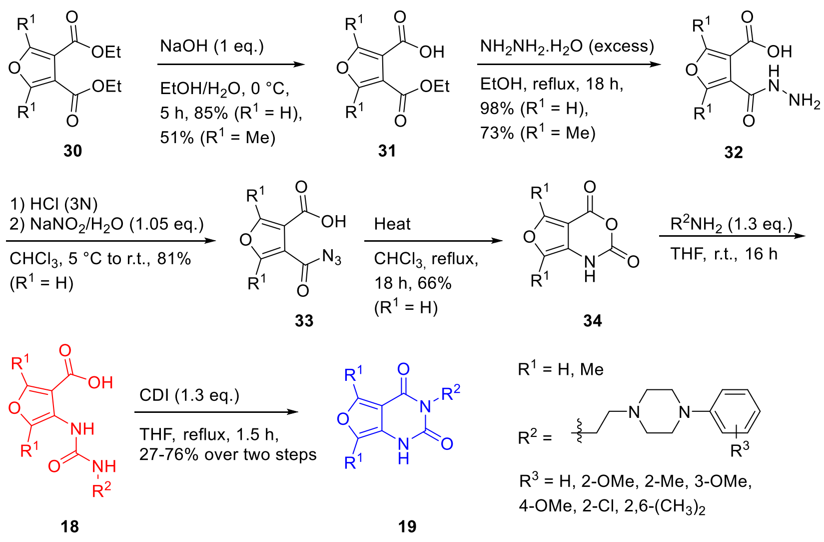
Scheme 3. The synthesis of furo[3,4-d]pyrimidine-2,4-dione

A similar method, starting from the dimethyl ester, was developed by Zhan and Yao. In this case, the dimethyl ester was only partially aminolyzed into the mono-amide, which was then transformed into amine **29** via a Hofmann rearrangement in good yield (Scheme 4). Hydrolysis of the ester and subsequent condensation with urea delivered the desired scaffold **6**.[30]



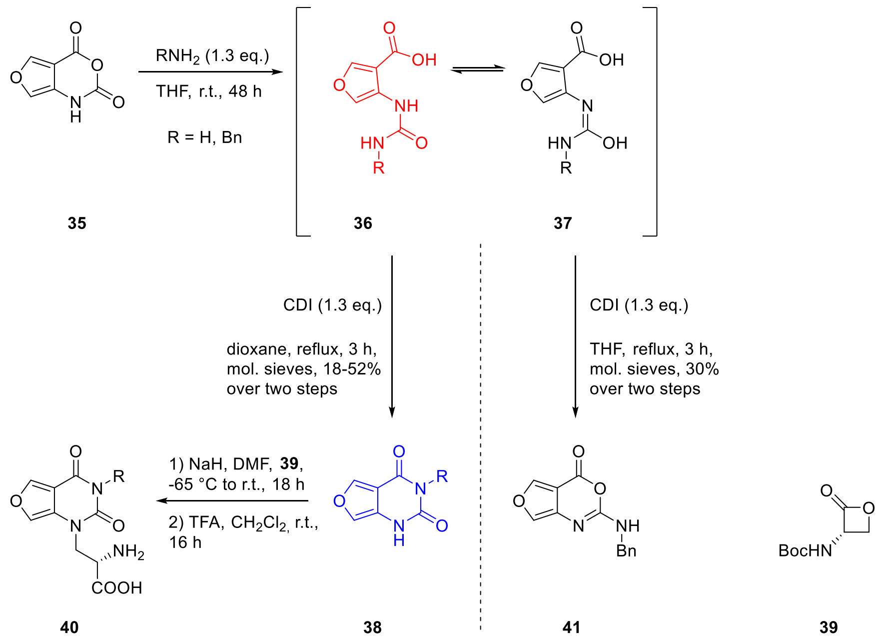
Scheme 4. The synthesis of furo[3,4-d]pyrimidine-2,4-dione

A third pathway starting from the dialkyl ester was described by Press and coworkers (Scheme 5). After mono-saponification of the diethyl ester **30** with sodium hydroxide, mono-ester **31** was treated with hydrazine to give hydrazide **32** in excellent yield. This hydrazide was converted into the corresponding acyl azide upon treatment with aqueous nitrous acid. This compound appeared to be stable but was nevertheless converted immediately into isatoic anhydride derivative **34** by heating in chloroform. This anhydride is crystalline and can be stored for a long time without any special precautions. Ring opening with various amines, followed by ring closure with CDI, yielded a library of furo[3,4-*d*]pyrimidinones **19**.[31,32]



*Scheme 5. Synthesis of N-3 functionalized furo[3,4-d]pyrimidine-2,4-diones **19***

Butini *et al.* followed the same strategy to synthesize furopyrimidinones **40** (Scheme 6). Interestingly, they observed that the ureid **36**, which was formed after ring opening of the isatoic anhydride derivative **35** with an amine, was in equilibrium with its enol tautomer **37**. By performing the subsequent ring closure in either THF or dioxane as a solvent, two different isomers **38** and **41** were obtained. Alkylation of **38** at *N*-1, followed by deprotection, eventually resulted in compounds **40**.[33]



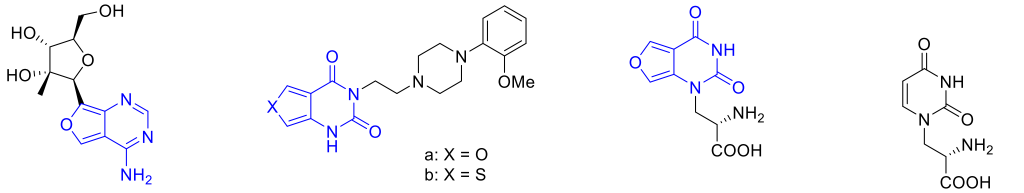
*Scheme 6. Synthesis of N-1/N-3 functionalized furo[3,4-d]pyrimidine-2,4-diones **40***

1.2 Biological activity

Nucleoside analogue **25** (Figure 5, Scheme 2) was reported in a patent on new antiviral compounds, more specifically against *Flaviviridae*. One of the members of this family of viruses is the hepatitis C virus. A hepatitis C virus nonstructural protein 5B (HCV NS5B) polymerase assay was described, but no test results were included. Therefore, it is not known if compound **25** possesses antiviral activity.[28]

Compound **42a** (Figure 5, Scheme 5) possesses antihypertensive activity in rats and is a potent α_1-adrenergic antagonist with a K_i value of 5.8 nM. One of the important functions of α_1-adrenergic receptors is mediation of smooth muscle contraction. Remarkably, **42a** is ninefold less potent than its corresponding thieno[3,4-*d*]pyrimidine-2,4-dione analogue **42b** (K_i = 0.64 nM).[32]

Furopyrimidinone **43** (Figure 5, Scheme 6) is an analogue of (S)-Willardiine **44**, a heterocyclic amino acid which is found in the seeds of several species of *Acacia* and *Mimosa* and which is a potent α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor agonist. The AMPA, *N*-methyl-D-aspartate (NMDA) and kainic acid (KA) receptors are three types of ionotropic glutamate receptors. These receptors play a key role in synaptic transmission in the CNS, and selective agonists could help in the understanding of their role in learning and memory formation and possible associations with neurological disorders such as Alzheimer's, Parkinson's and Huntington's diseases. Compound **43** was tested *in vitro* but possesses only very weak binding affinity for the AMPA and KA glutamate receptors.[33]



Nucleoside analogue
Biocryst Pharmaceuticals
(2010)
25

Antihypertensive agent
Academic
(1989)
42

AMPA/KA receptor agonist
Academic
(2008)
43

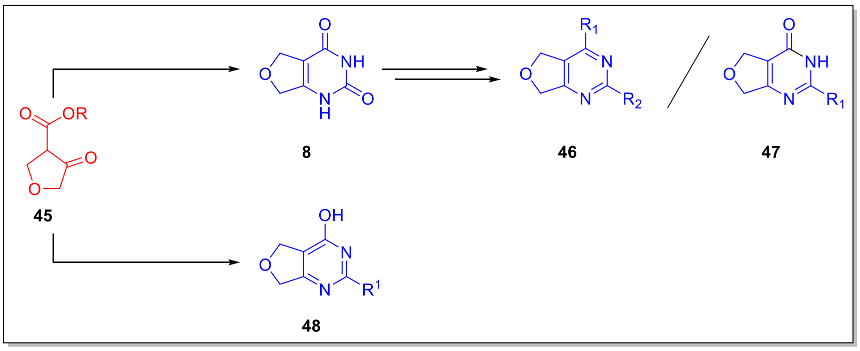
Natural product
(S)-Willardiine
44

Figure 5. Biologically active furo[3,4-d]pyrimidine derivatives

2 Synthesis and biological activity of 5,7-dihydrofuro[3,4-*d*]pyrimidines

2.1 Synthesis of 5,7-dihydrofuro[3,4-*d*]pyrimidines

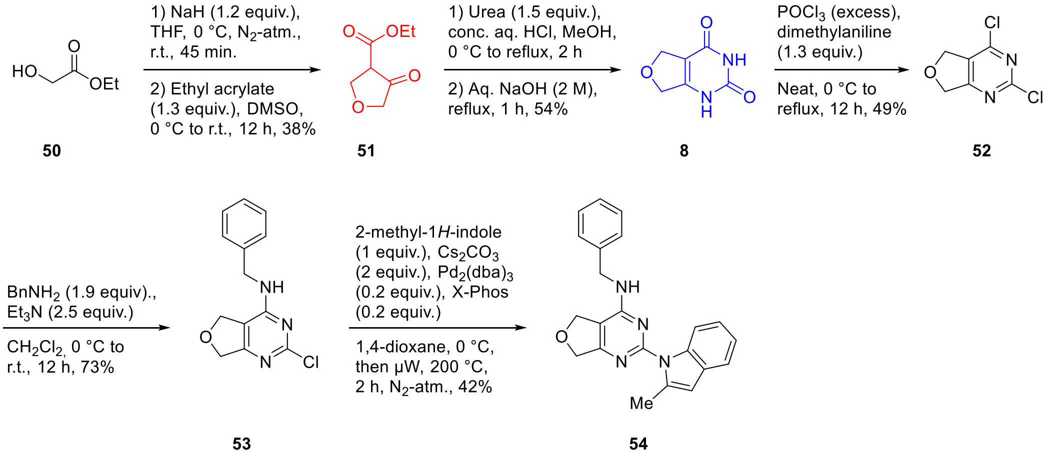
Dihydrofuro[3,4-*d*]pyrimidines have been synthesized and described in large numbers (>100 references). Therefore, the scope in the present overview was limited to those structures without substituents or functionalizations on the dihydrofuran ring. All of the reported syntheses of this scaffold started from alkyl 4-oxotetrahydrofuran-3-carboxylate **45** (Scheme 7). The pyrimidine annulation was then effectuated by reaction with an appropriate ureid or amidine compound.



Scheme 7. Synthesis of 5,7-dihydrofuro[3,4-d]pyrimidines by annulation of pyrimidine rings

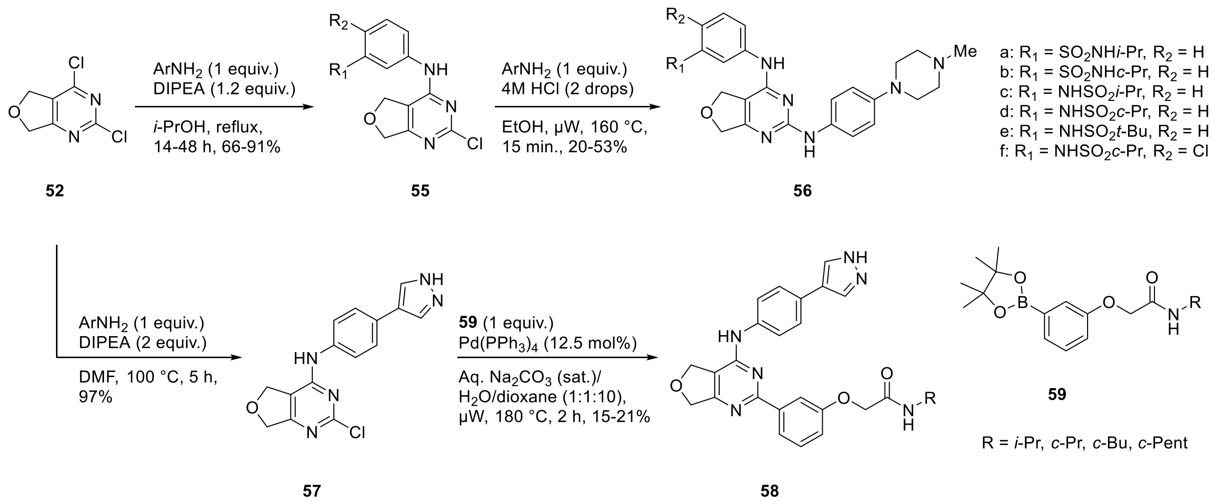
2.1.1 Scaffold construction by reaction of a ketoester with urea

Reacting ethyl 2-hydroxyacetate **50** with ethyl acrylate in the presence of sodium hydride delivered ethyl 4-oxotetrahydrofuran-3-carboxylate **51** in low yield (Scheme 8). Condensation with urea in a mixture of concentrated aqueous hydrochloric acid and methanol under reflux conditions resulted in dihydrofuropyrimidinone **8**. Chlorination with phosphorus oxychloride afforded chloropyrimidine **52**. This compound was selectively functionalized at the 4-position by reaction with benzylamine and at the 2-position by reaction with 2-methyl-1*H*-indole under Buchwald-Hartwig conditions to afford compound **54**.[34,35]



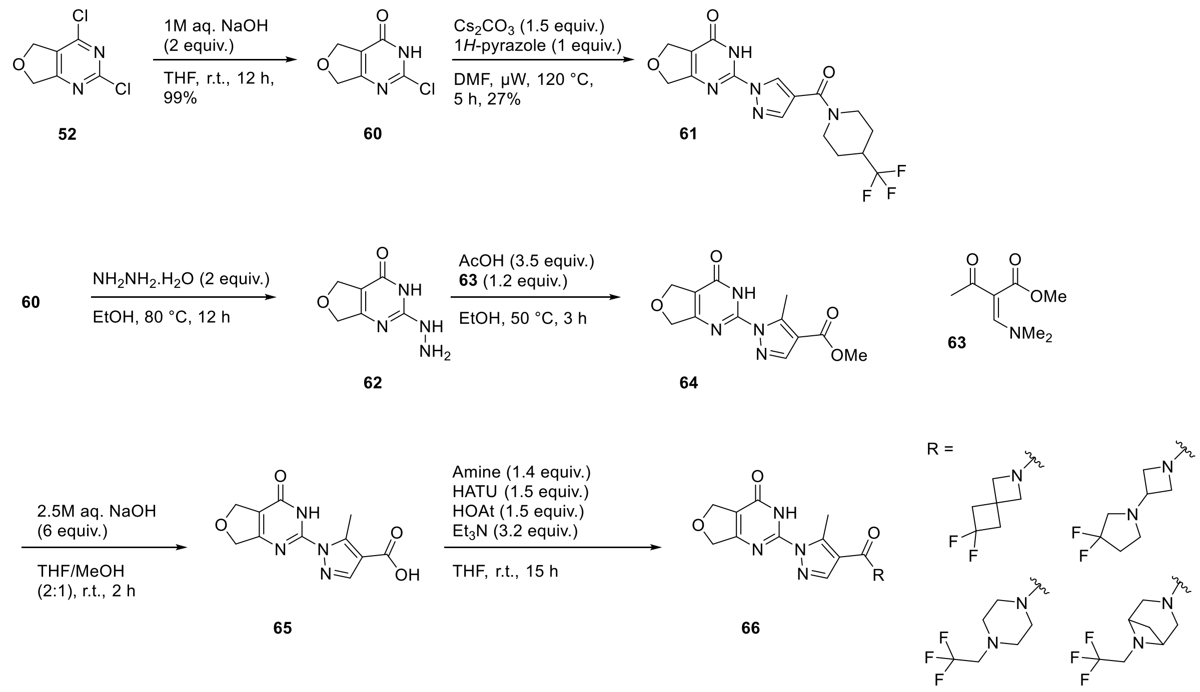
Scheme 8. Synthesis of 5,7-dihydrofuro[3,4-d]pyrimidine-2,4-dione and further decoration of the scaffold

Disubstituted dihydropyrimidines **56** were synthesized from the same chloropyrimidine **52** again by using the difference in reactivity of the chlorines at the 2- and 4-position (Scheme 9). Both positions were substituted with anilines. This time, substitution at the 2-position was achieved by acid instead of palladium catalysis (*vide supra*).[36] In a similar fashion, compounds **58** were synthesized. The chlorine at position 4 of **52** was substituted again with an appropriate aniline, and the 2-chlorine was then functionalized by a Suzuki coupling to obtain the 2,4-disubstituted structures **58** in very low yields.[37]



Scheme 9. Decoration of the 2,4-dichlorofuro[3,4-d]pyrimidine scaffold via two different pathways

Furthermore, 2,4-dichloropyrimidine **52** was converted quantitatively to the 2-chloropyrimidin-4-one **60** by treatment with sodium hydroxide (Scheme 10). Reaction of this chloropyrimidinone with an appropriate pyrazole afforded **61** in low yield. The methylpyrazole-functionalized compounds **66** were constructed differently. First, the chlorine was substituted with hydrazine. Next, the methylpyrazole ring was constructed by reaction of the hydrazine substituent with enamine **63**. After saponification of the ester and subsequent amide coupling, products **66** were obtained.[38]

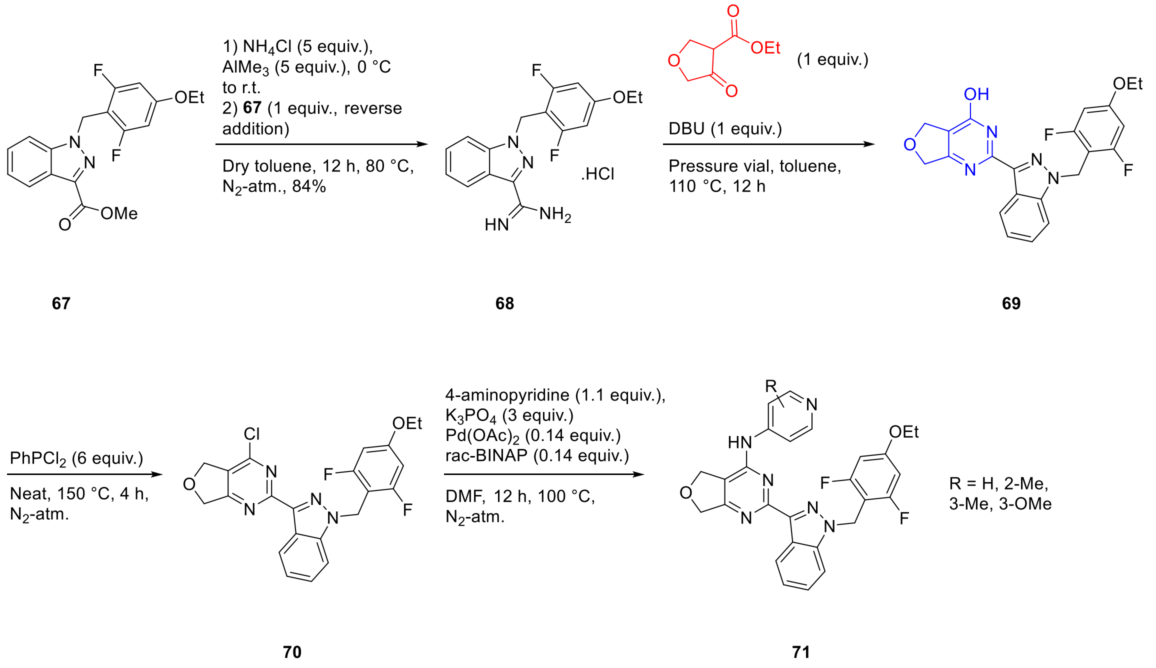


*Scheme 10. Decoration of the 2,4-dichlorofuro[3,4-d]pyrimidine scaffold towards the structures **66***

2.1.2 Scaffold construction by reaction of a ketoester with an amidine

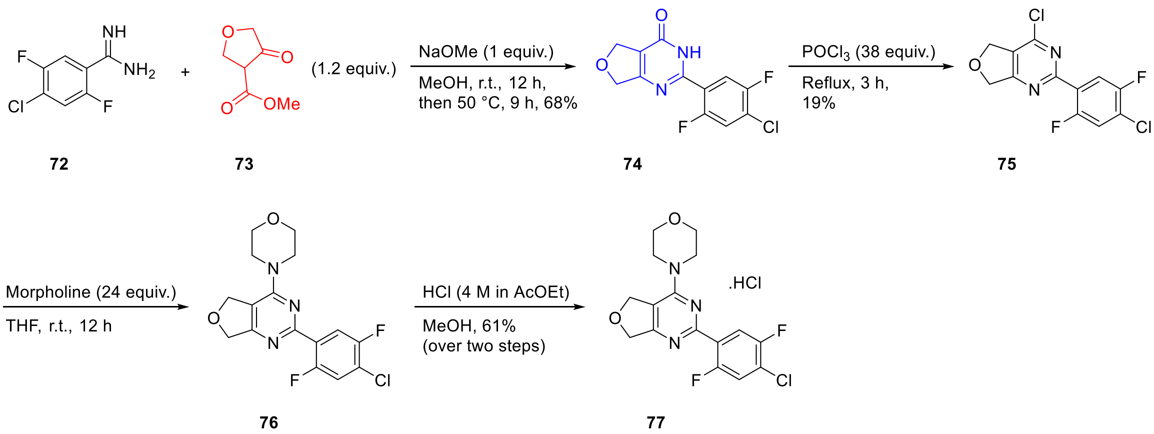
The dihydrofuropyrimidine scaffold has also been constructed directly by reaction of ketoester **51** with a variety of amidines. Different bases were employed for the condensation reaction, and different chlorinating reagents were used for the subsequent derivatization.

Reaction of ester **67** with ammonium chloride and trimethylaluminium afforded amidine **68** in good yield (Scheme 11). This amidine was then condensed with the ketoester and DBU as a base to give the 4-hydroxydihydrofuropyrimidine **69**. Chlorination was performed with phenylphosphonous dichloride, and the chloropyrimidine then underwent Buchwald-Hartwig coupling with various 4-aminopyridines to give compounds **71**.[39]



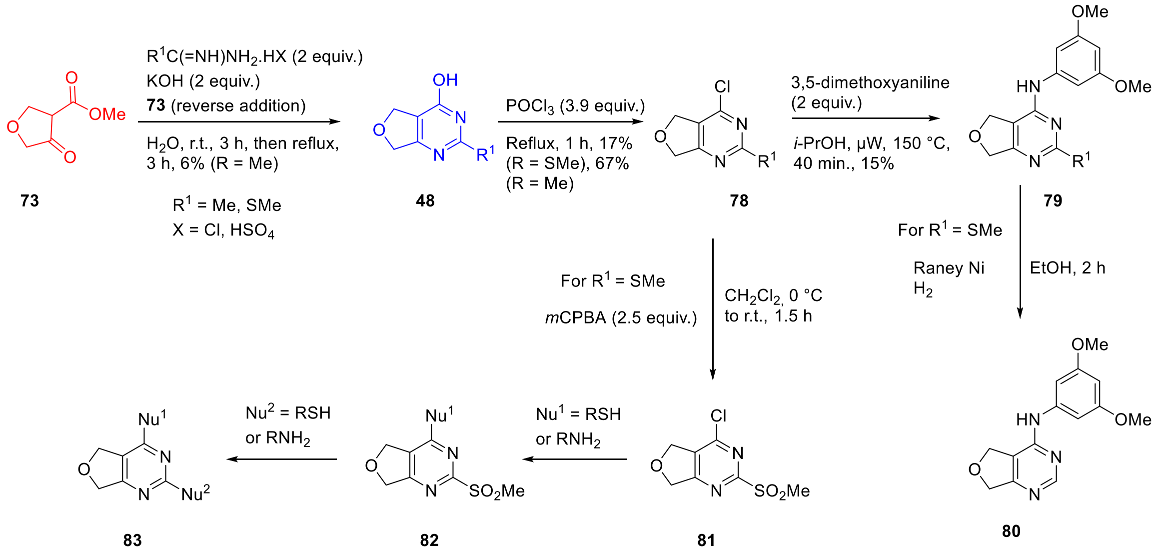
*Scheme 11. Construction of the 5,7-dihydrofuro[3,4-d]pyrimidine scaffold and further modification towards the structures **71***

The same approach was used to construct dihydrofuropyrimidines **77** (Scheme 12). Here, sodium methoxide was used as a base in the coupling of the amidine (**72**) and ketoester (**73**) fragments. Chlorination of the resulting dihydrofuropyrimidinone **74** took place in refluxing phosphorus oxychloride but only proceeded in low yield. Substitution of the 4-position with morpholine and subsequent addition of hydrochloric acid afforded the hydrochloric acid salt **77** in acceptable yield.[40]



Scheme 12. Construction of the 5,7-dihydrofuro[3,4-d]pyrimidine scaffold and further decoration

When S-methylisothiourea was used as the amidine source, a thiomethyl substituent was obtained at the 2-position of the dihydrofuropyrimidine scaffold **48** (Scheme 13). This proved to be a versatile substituent, as it can be oxidized towards the sulfone, removed by Raney nickel-catalyzed hydrogenolysis or it can be retained as such. For the sulfone-containing chloropyrimidine **81**, both the chlorine and the sulfone group were replaced by various nucleophiles affording disubstituted dihydrofuropyrimidines **83.**[41]



Scheme 13. Different methods for the decoration of the 5,7-dihydrofuro[3,4-d]pyrimidine scaffold

2.2 Biological activity

Compound **54** (Figure 6, Scheme 8) is an *in vitro* inhibitor of protein p97 (an ATPase of the AAA family) with an IC_{50} value below 300 nM. The p97 protein is involved in a broad array of cellular processes including the fusion of homotypic membranes, protein degradation and activation of membrane-bound transcription factors. Since its function is essential for continued cellular viability, inhibitors of p97 have an antiproliferative effect.[42] The possible antiproliferative activity of **54** was not tested in the cited study.[34]

Compound **56a** (Figure 6, Scheme 9) is an *in vitro* inhibitor of bromodomain 4 (BRD4) kinase with an IC_{50} value of 0.22 µM. BRD kinases are essential for the recognition of acetylated lysine residues of histones during transcriptional activation. Therefore, BRD4 kinase inhibitors could be useful as anticancer agents.[36] Compound **84** (Figure 6, Scheme 9) is an *in vitro* glucose uptake inhibitor and inhibits glycolysis in HT1080 fibrosarcoma cells and *P. falciparum*-infected RBCs with IC_{50} values between 10 and 100 nM.[37]

Compound **61** (Figure 6, Scheme 10) was shown to be a very potent *in vitro* inhibitor of sepiapterin reductase (SPR), with an IC_{50} value of 6 nM. SPR is a crucial enzyme in the *de novo* biosynthesis of tetrahydrobiopterin (BH_4), an enzyme cofactor for various aromatic amino acid hydroxylases, nitric oxide synthases and alkylglycerol monooxygenase. SPR inhibitors could be used in the treatment of acute and chronic pain or for anti-inflammation and immune cell regulation purposes.[38]

Compound **85** (Figure 6, Scheme 11) was found to be a very potent *in vitro* BUB1 kinase inhibitor with an IC_{50} value of 7 nM. BUB1 kinase is a mitotic checkpoint protein and complete inhibition of this checkpoint could result in induction of apoptosis. **85** showed antiproliferative activity against HeLa tumor cells with an IC_{50} value of 1.5 µM.[39]

Compound **77** (Figure 6, Scheme 12) is an *in vitro* G protein-coupled receptor 119 (GPR119) agonist with an EC_{50} value of 4.1 µM. GPR119 has been reported to play a role in regulating glucose-dependent insulin secretion (GDIS) from pancreatic β-cells. Therefore, GPR119 agonists have potential for the treatment of type 2 diabetes. Interestingly, the corresponding dihydrothieno[3,4-*d*]pyrimidine structure of **77** performed better as it had an EC_{50} value of 150 nM in the same assay.[40]

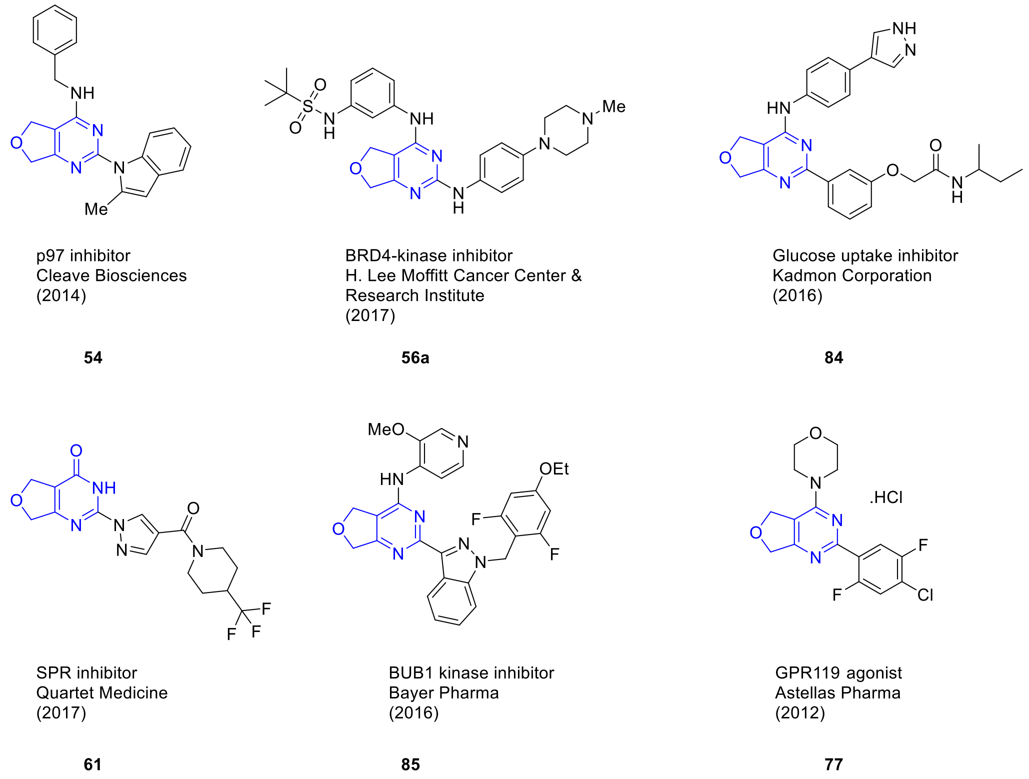


Figure 6. Biologically active 5,7-dihydrofuro[3,4-d]pyrimidine derivatives

Compound **86** (Figure 7, Scheme 13) acts as an *in vitro* caspase cascade activator with respective EC_{50} values of 61 nM in the human breast cancer cell line T-47D, 5.5 µM in human colon carcinoma cell line HCT116 and 4.7 µM in the human hepatocellular carcinoma cell line SNU398. The compound also inhibits cell proliferation for these cell lines *in vitro*. Caspases are a family of protease enzymes that play essential roles in programmed cell death and inflammation.[41]

Additional activity data is also presented for a number of structures which were synthesized using the methodologies described in the preceding paragraphs. Compound **87** (Figure 7) is a selective inhibitor of Rho-associated protein kinase (ROCK2) with an IC_{50} value of 10 nM and could be used in the treatment of graft versus host disease (GVHD).[43,44] Product **88** (Figure 7) is an inhibitor of the human phosphodiesterase hPDE4B. PDE4 inhibitors are expected to be promising anti-inflammatory agents for the treatment of asthma, chronic obstructive pulmonary disease (COPD) and other inflammatory diseases. Compound **88** had only a low activity of 2.2 µM compared to its respective dihydrothieno[3,4-*d*]pyrimidine analogue.[45] This exemplifies again that the substitution of oxygen by sulfur can have a dramatic effect on the biological activity for this scaffold.

Compounds **89a-d** (Figure 7) were tested against 14 different phytopathogenic fungi. For 9 strains, one or more of the structures possessed significant antifungal activity. The potency rank order depended on the fungal strain, but in general compound **89c** performed best with significant activity against 7 strains.[46] Compounds **90** are *in vitro* inhibitors of mTOR kinase with K_i values between 6-10 nM. They also inhibit cell proliferation of PC3 human prostate cancer cells *in vitro* with EC_{50} values between 28-331 nM.[47] Compounds **91a-b** are inhibitors of transforming growth factor-beta (TGF-β) with IC_{50} values of 3.1 and 2.25 μM, respectively. TGF-β modulates cell growth and differentiation, embryonic and bone development, extracellular matrix formation, hematopoiesis, immune and inflammatory responses. Therefore, TGF-β inhibitors could be useful in treating conditions such as fibrotic, pulmonary and oncologic disorders.[48]

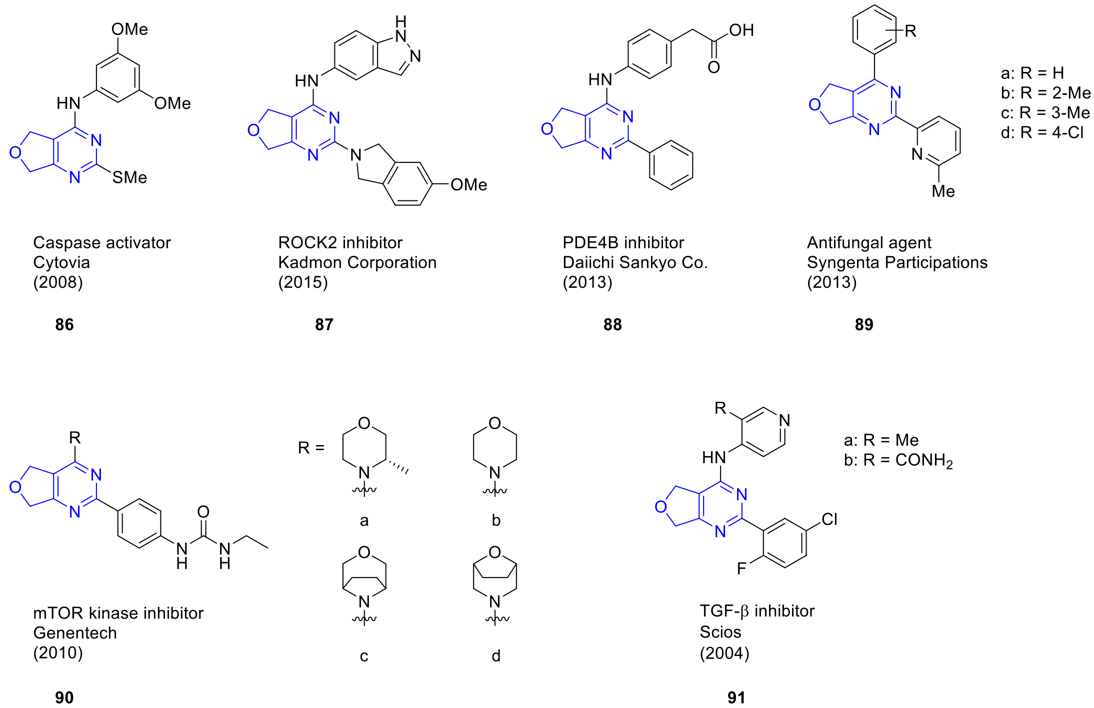


Figure 7. Biologically active 5,7-dihydrofuro[3,4-d]pyrimidine derivatives

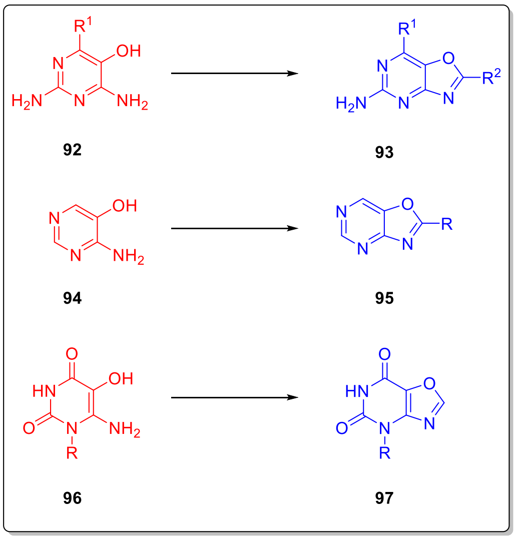
3 Synthesis and biological activity of oxazolo[4,5-*d*]pyrimidines

Oxazolo[4,5-*d*]pyrimidines have been investigated to a limited extent only. One reason is the lack of a versatile building block such as 5,7-dichlorooxazolo[4,5-*d*]pyrimidine. Scaffold construction is generally achieved by oxazole ring annulation on a functionalized pyrimidine or by pyrimidine ring annulation on a functionalized oxazole.

3.1 Synthesis of oxazolo[4,5-*d*]pyrimidines

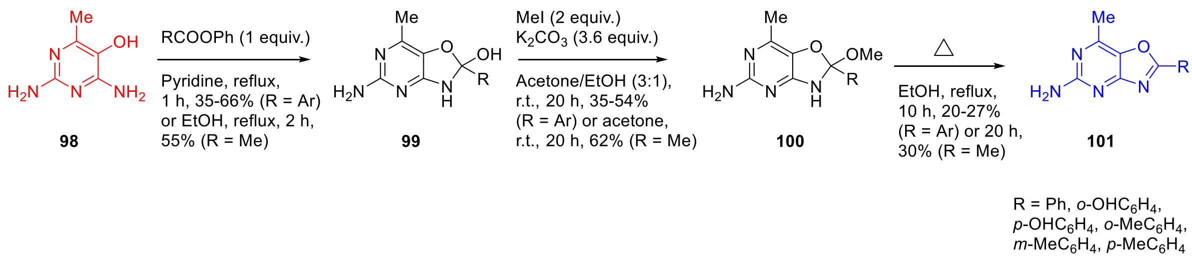
3.1.1 Scaffold construction starting from a functionalized pyrimidine

Oxazolo[4,5-*d*]pyrimidines can be synthesized starting from a 4-amino-5-hydroxypyrimidine by annulation of an oxazole ring (Scheme 14). The carbon source for the C(2) of the scaffold can be an ester, orthoester, thioimidate, carboxylic acid or formamide.



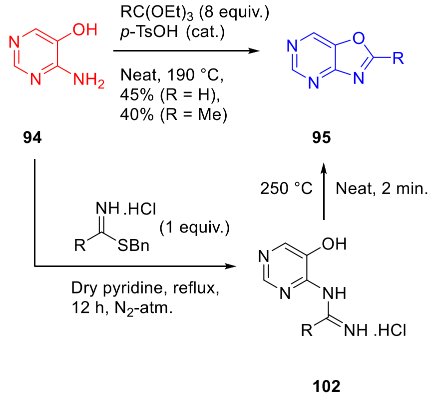
Scheme 14. Oxazolo[4,5-d]pyrimidines by annulation of oxazole rings

Kato *et al.* were the first to report the synthesis of oxazolo[4,5-*d*]pyrimidines. They discovered that the reaction of 2,4-diamino-5-hydroxy-6-methylpyrimidine **98** with phenyl esters delivered hemi-aminals **99**, which were isolated as stable compounds at room temperature in moderate yields (Scheme 15). When these hemi-aminals were heated to 110-140 °C, ring closure to the oxazolopyrimidines **101** was observed, but also ring opening to the ester and the amide. To reduce these side reactions, the hemi-aminals were first methylated with iodomethane and then heated. The desired oxazolo[4,5-*d*]pyrimidines **101** were still only obtained in low yields.[49,50]



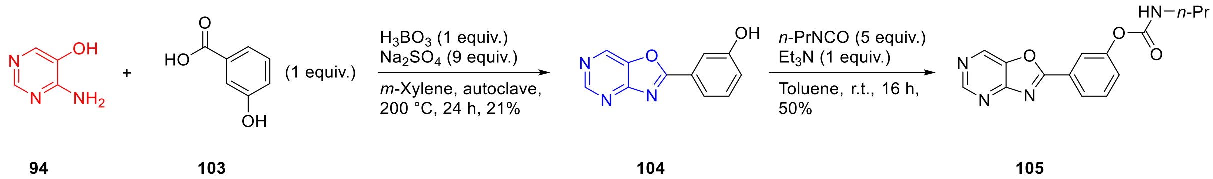
Scheme 15. Reaction of 2,4-diamino-5-hydroxy-6-methylpyrimidine with phenyl esters

A more direct approach was developed by Doise and coworkers. They obtained the desired scaffold in one step by condensing aminopyrimidinol **94** with excess orthoester and a catalytic amount of *para*-toluenesulfonic acid at high temperature (Scheme 16).[51] In later work, a thiobenzyl imidate proved a suitable alternative to an orthoester. This lead to an open intermediate **102**, which was ring closed by heating at 250 °C for two minutes (Scheme 16). No yields were reported for the latter approach.[52]



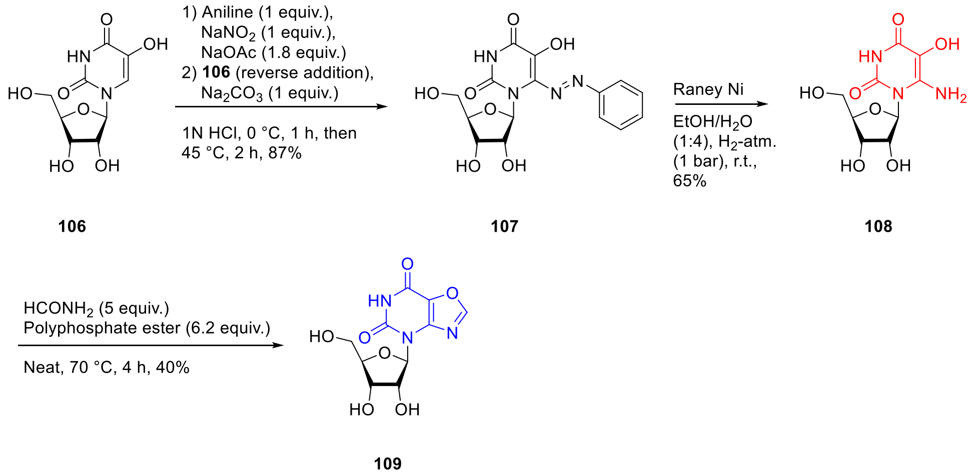
Scheme 16. Condensation of an aminopyrimidinol with excess orthoester or a thiobenzyl imidate

Besides thioimidates and orthoesters, a carboxylic acid can also be used as a source of the C(2) carbon atom of the oxazolopyrimidine scaffold. When 4-aminopyrimidin-5-ol **94** was reacted with 3-hydroxybenzoic acid under harsh reaction conditions, oxazolopyrimidine scaffold **104** was obtained in low yield (Scheme 17). Further reaction of the phenolic hydroxyl group with *n*-propylisocyanate afforded carbamate **105**.[53]



Scheme 17. Condensation of an aminopyrimidinol with a carboxylic acid

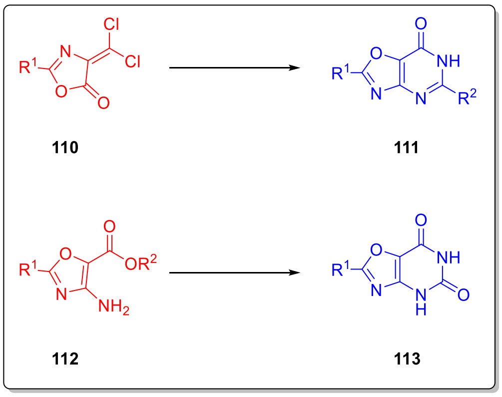
Formamide can also be used as the source of the C(2) carbon atom. Starting from 5-hydroxyuridine **106**, a diazocoupling reaction gave diazobenzene derivative **107** in very good yield (Scheme 18). Raney nickel-catalyzed hydrogenolysis followed by ring closure with formamide and polyphosphate ester as dehydrating agent finally delivered the nucleoside analogue **109** in low yield.[54]



Scheme 18. Condensation of an aminopyrimidinol with formamide

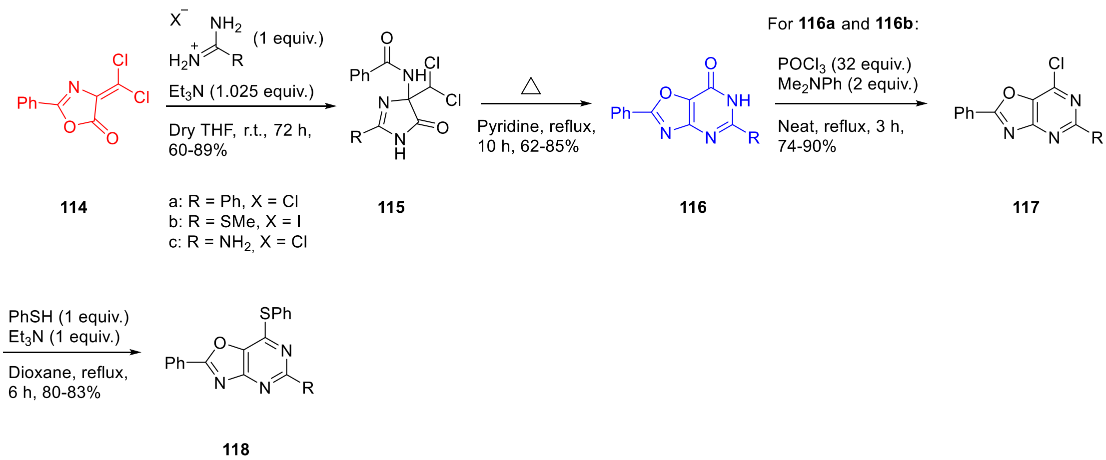
3.1.2 Scaffold construction starting from a functionalized oxazole

Oxazolo[4,5-*d*]pyrimidines have also been synthesized starting from a functionalized oxazole by annulation of a pyrimidine ring (Scheme 19).



Scheme 19. Oxazolo[4,5-d]pyrimidines by annulation of a pyrimidine ring

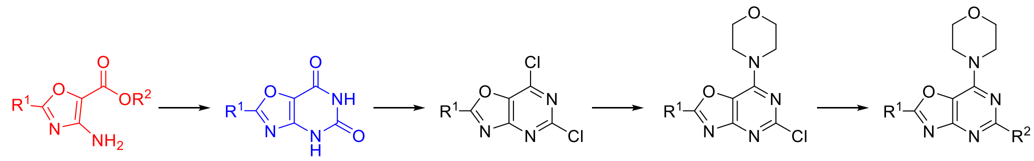
Reaction of 1,3-oxazol-5(4*H*)-one **114** with various amidines gave imidazolones **115** in good yields (Scheme 20). These intermediates were converted to the oxazolo[4,5-*d*]pyrimidin-7(6*H*)-ones **116** by heating in refluxing pyridine. In the case of a phenyl or thiomethyl substituent at the 5-position of this scaffold, chlorination with phosphorus oxychloride was achieved in good yields. The obtained chloropyrimidines **117** were then reacted with thiophenol to afford final structures **118** in very good yields. The authors did not mention whether they also attempted to react **116c** (R = NH_2) with phosphorus oxychloride.[55]



Scheme 20. Condensation of dichloromethylidene 1,3-oxazol-5(4H)-ones with amidines

The synthesis of twenty-eight new C(2)/C(4)-functionalized oxazolo[4,5-*d*]pyrimidines **121** was reported by Cmiljanovic *et al.* (Scheme 21). Unfortunately, the applied synthetic methods and characterization of these compounds was not described in detail. The only structures of Scheme 21 which were unambiguously reported are the final structures **121**. According to the reported general reaction scheme, the starting material was 4-aminooxazole **112** with an ester group at the 5-position and optionally a substituent at the 2-position. This oxazole was cyclized to afford oxazolo[4,5-

d]pyrimidin-5,7(4*H*,6*H*)-dione **113**. Chlorination of this compound then lead to 5,7-dichloropyrimidine **119**, which was subsequently substituted at the 7- and 5-positions to obtain the final structures **121**.[56]



112 **113** **119** **120** **121**

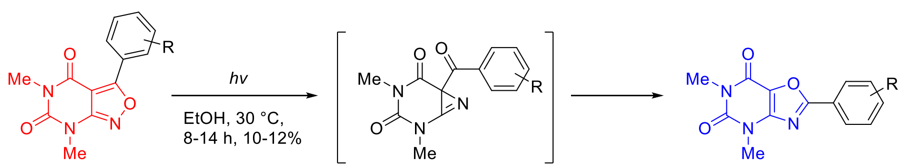
R^1 = H, (4-methylpiperazin-1-yl)methyl, (4-(methylsulfonyl)piperazin-1-yl)methyl

R^2 = 1*H*-indol-4-yl, 1*H*-indol-5-yl, 1*H*-indazol-4-yl, 1*H*-indazol-5-yl, 1*H*-indazol-6-yl, 1*H*-benzo[d]imidazol-4-yl, 1*H*-benzo[d]imidazol-5-yl, 9*H*-carbazol-2-yl, 6-acetylaminopyridin-3-yl, *N*-acetyl-4-phenyl, 6-morpholinopyridin-3-yl, phenol-3-yl, 6-aminopyridin-3-yl, 2-aminopyrimidin-5-yl, 2-amino-4-trifluoromethylpyridin-5-yl, 2-amino-4-trifluoromethylpyrimidin-5-yl

Scheme 21. Annulation of pyrimidines to 1,3-oxazol-5(4H)-ones

3.1.3 Scaffold construction by rearrangement of isoxazolo[3,4-*d*]pyrimidines

Nishigaki and coworkers investigated the photochemical behavior of 3-arylisoxazolo[3,4-d]pyrimidines **122** and observed a photorearrangement upon irradiation with a 100W high-pressure mercury lamp yielding the corresponding 2-aryloxazolo[4,5-*d*]pyrimidines **124** in very low yields (Scheme 22). Based on a previous mechanistic study on the photorearrangement of isoxazoles to oxazoles, which proceeds *via* an azirine intermediate, they assumed that this ring transformation also occurs via the initial formation of an azirine intermediate, **123**, followed by recyclization to **124**.[57]



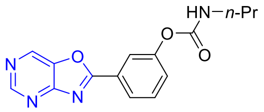
122 **123** **124**

Scheme 22. Photochemically induced rearrangement of 3-arylisoxazolo[3,4-d]pyrimidines

3.2 Biological activity

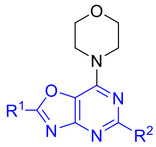
Compound **105** (Figure 8, Scheme 17) is an inhibitor of fatty acid amide hydrolase (FAAH) with an IC_{50} value of 4.5 µM and also inhibits monoglyceride lipase (MGL) by 18% at 100 µM. FAAH and MGL are the main enzymes responsible for the hydrolysis of the two major endogenous cannabinoids *N*-arachidonoylethanolamide (AEA) and 2-arachidonoylglycerol (2-AG). These cannabinoids activate the cannabinoid receptors CB1 and CB2, which induces biological effects such as relief of pain and anxiety. Thus, inhibitors of FAAH and MGL could have anxiolytic and analgesic activity.[53]

Compounds **121** (Figure 8, Scheme 21) were reported in a large study on protein kinase B (PKB) inhibitors. PKB plays a key role in multiple cellular processes such as glucose metabolism, apoptosis, cell proliferation, transcription and cell migration. A PKB screening assay protocol was described in the publication, but it is not clear whether these compounds were included in the screen, and no biological results are thus available for these structures.[56]



FAAH inhibitor
Academic
(2009)

105



Academic
(2009)

121

R^1 = H, (4-methylpiperazin-1-yl)methyl, (4-(methylsulfonyl)piperazin-1-yl)methyl

R^2 = 1*H*-indol-4-yl, 1*H*-indol-5-yl, 1*H*-indazol-4-yl, 1*H*-indazol-5-yl, 1*H*-indazol-6-yl, 1*H*-benzo[d]imidazol-4-yl, 1*H*-benzo[d]imidazol-5-yl, 9*H*-carbazol-2-yl, 6-acetylaminopyridin-3-yl, *N*-acetyl-4-phenyl, 6-morpholinopyridin-3-yl, phenol-3-yl, 6-aminopyridin-3-yl, 2-aminopyrimidin-5-yl, 2-amino-4-trifluoromethylpyridin-5-yl, 2-amino-4-trifluoromethylpyrimidin-5-yl

Figure 8. Oxazolo[4,5-d]pyrimidines of biological interest

4 Synthesis and biological activity of oxazolo[5,4-*d*]pyrimidines

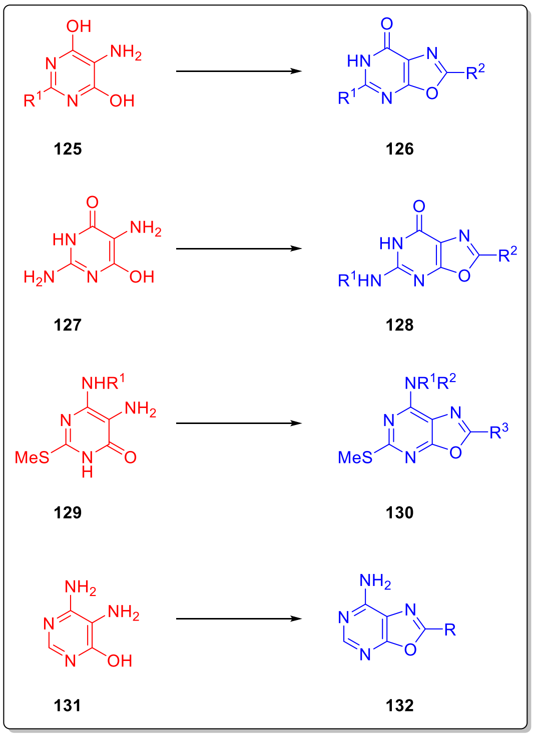
The synthesis of oxazolo[5,4-*d*]pyrimidines has been described to a much larger extent than the aforementioned oxazolo[4,5-*d*]pyrimidines. Generally, this scaffold can be constructed starting from either a functionalized pyrimidine or oxazole.

4.1 Synthesis of oxazolo[5,4-*d*]pyrimidines

4.1.1 Scaffold construction starting from a functionalized pyrimidine

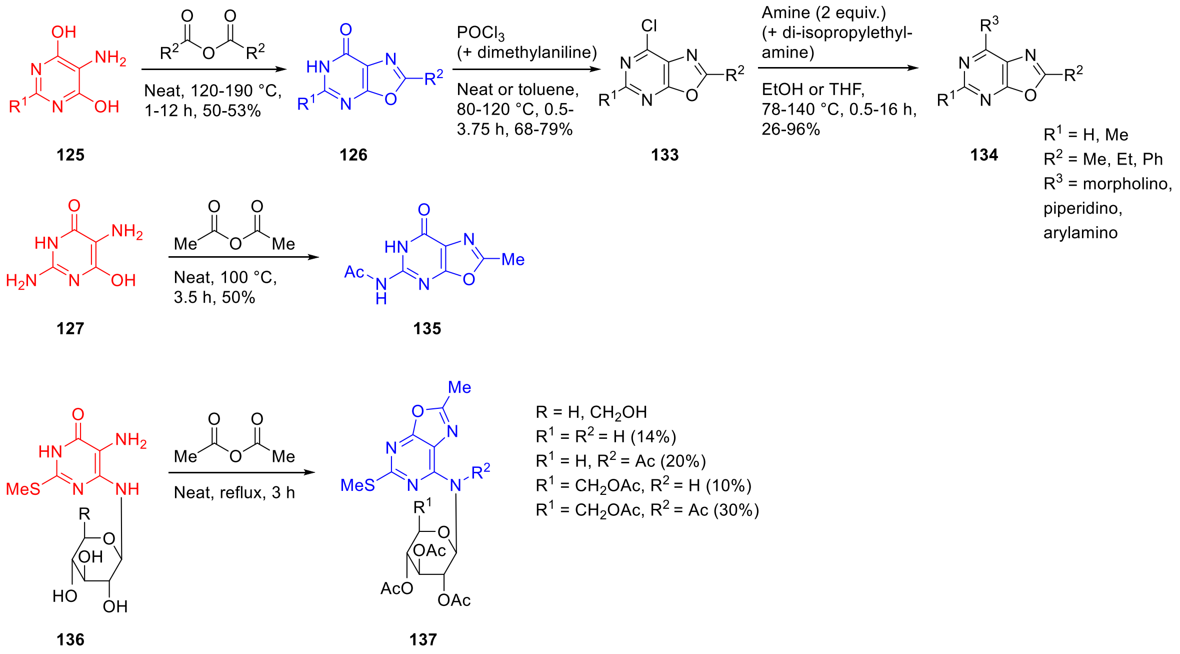
4.1.1.1 Starting from a 5-amino-4(,6)-(di)hydroxypyrimidine

Possible building blocks to construct oxazolo[5,4-*d*]pyrimidines in a single step are 5-aminopyrimidines with a hydroxyl group or a lactam-type carbonyl group on the 4- and/or 6-position (Scheme 23). The carbon source for the C(2) of the resulting oxazolopyrimidine can originate from an anhydride, nitrile or trifluoromethyl moiety.



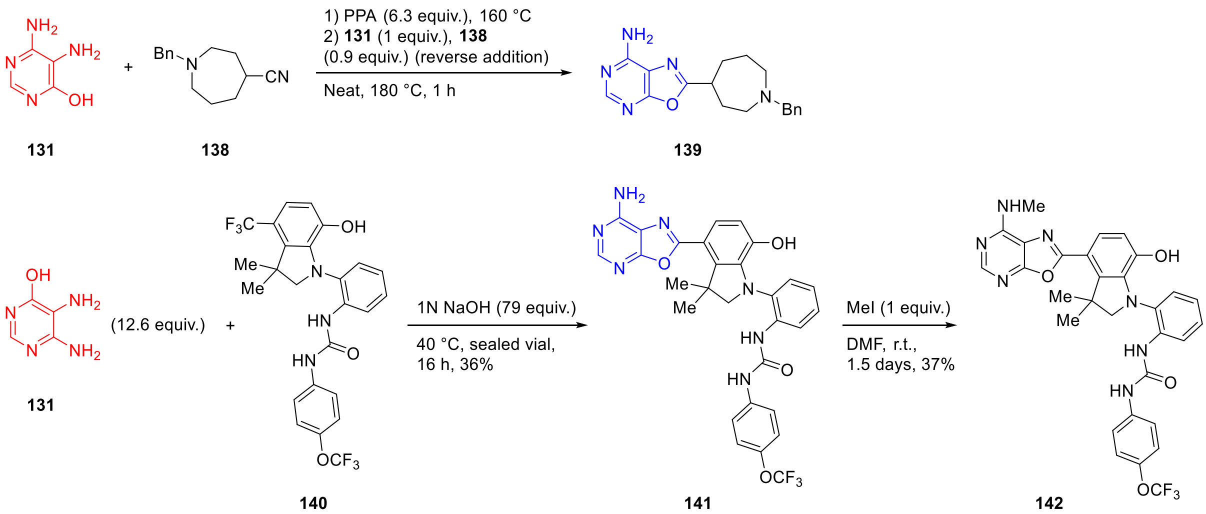
Scheme 23. *Oxazolo[5,4-d]pyrimidines by annulation of oxazole rings to 5-amino-4-hydroxypyrimidines*

Reaction of acetic, propionic or benzoic anhydride with 5-amino-4,6-dihydroxypyrimidine **125** afforded oxazolo[5,4-*d*]pyrimidinones **126** in moderate yields (Scheme 24). Subsequent chlorination with phosphorus oxychloride and reaction with morpholine, piperidine or various arylamines gave the final oxazolo[5,4-*d*]pyrimidines **134**.[58,59] Similarly, when diaminopyrimidinol **127** was heated in acetic anhydride, oxazolopyrimidinone **135** was obtained in moderate yield.[60] When the 6-hydroxyl group of the substituted pyrimidine was replaced by a secondary amino group, oxazole ring annulation occurred between the 4-carbonyl group and the 5-amino group of the pyrimidine ring. Treatment of amino(glycosylamino)pyrimidinones **136** with acetic anhydride under reflux gave (glycosylamino)oxazolopyrimidines **137** in low yield.[61]



Scheme 24. Condensation of 5-amino-4-hydroxypyrimidines with anhydrides

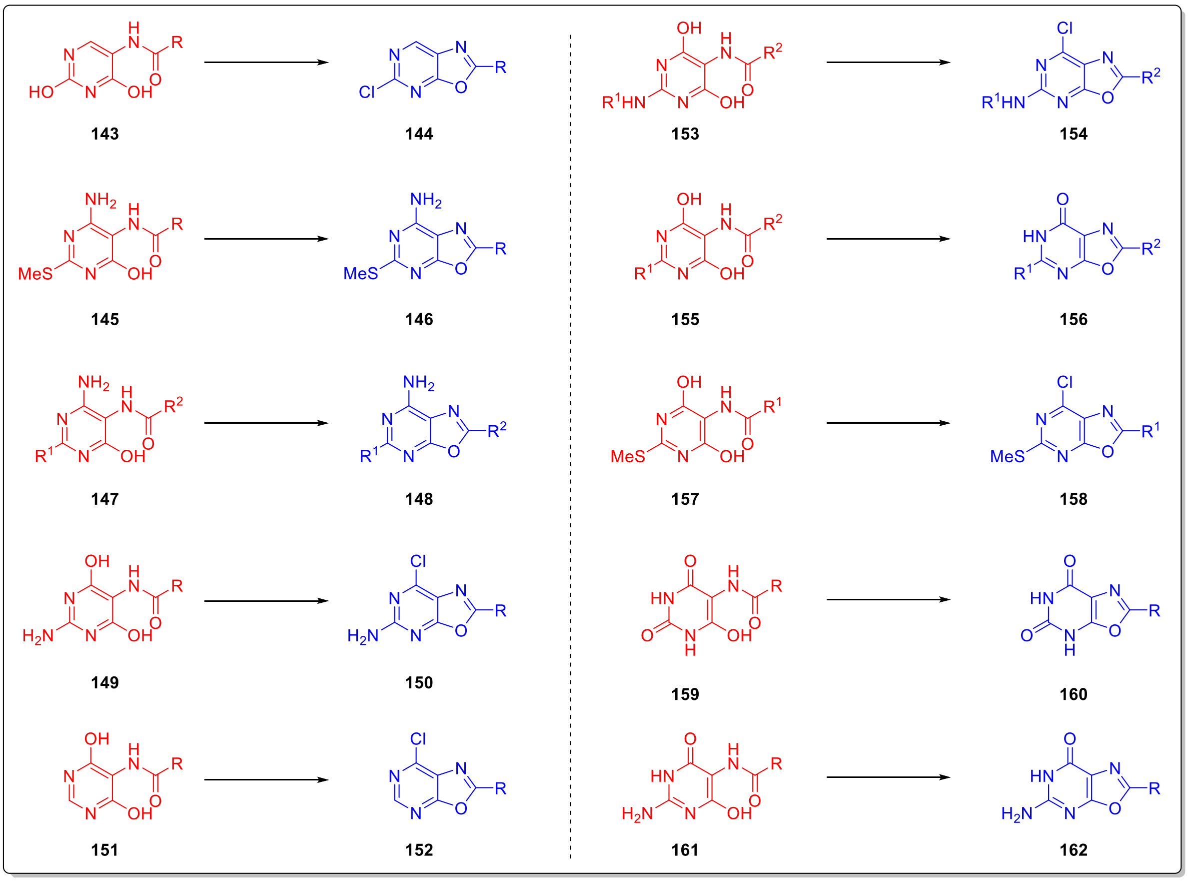
As mentioned before, a nitrile or trifluoromethyl moiety can also serve as the C(2) carbon source. Reaction of nitrile **138** with 5,6-diaminopyrimidin-4-ol **131** in hot polyphosphoric acid afforded 7-aminooxazolo[5,4-*d*]pyrimidine **139** (Scheme 25). The yield of this reaction was not reported.[62] Qiao and coworkers discovered that the trifluoromethyl group of structure **140** hydrolyzed to the corresponding carboxylic acid quite easily under basic conditions. This degradation pathway was used to replace the labile CF_3-group by more stable groups. Reaction of excess of 5,6-diaminopyrimidin-4-ol **131** with **140** under mild aqueous conditions gave the C(2)-substituted 7-aminooxazolo[5,4-*d*]pyrimidine **141** in low yield. Subsequent methylation afforded 7-methylaminooxazolopyrimidine **142** in low yield.[63]



Scheme 25. Condensation of 5-amino-4-hydroxypyrimidines with nitriles and trifluoromethyl groups

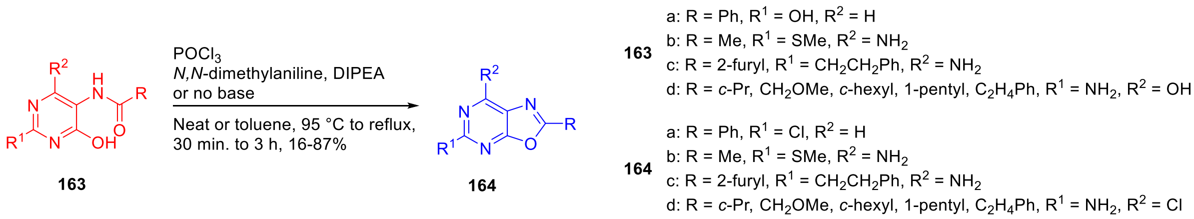
4.1.1.2 *Cyclization of a 5-acylamino-4(,6)-(di)hydroxypyrimidine*

One of the major approaches for the construction of oxazolo[5,4-*d*]pyrimidines is the cyclization of a 5-acylaminopyrimidine with a hydroxyl group or lactam-type carbonyl group on the 4- and/or 6-position (Scheme 26). Typical dehydrating agents are phosphorus oxychloride and polyphosphoric acid, but other reagents or techniques may also be used.



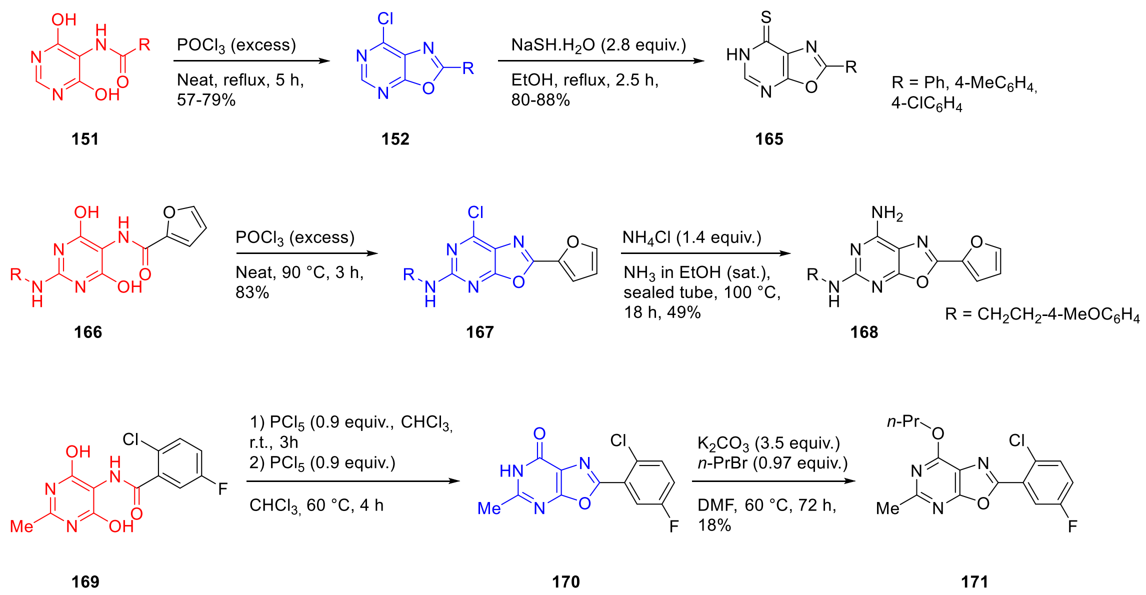
Scheme 26. Oxazolo[5,4-d]pyrimidines by cyclization of 5-acylamino-4-hydroxypyrimidines

5-Acylaminopyrimidine **163** was treated with excess *N*,*N*-dimethylaniline in phosphorus oxychloride under reflux conditions to afford oxazolopyrimidine **164** (Scheme 27). The very low yield of product **164b** was attributed to side reactions such as that of phosphorus oxychloride with the free 4-amino group yielding phosphorochloridate compounds.[64] Compound **163c**, which also possesses a 4-amino group, was ring-closed to oxazolopyrimidine **164c** in moderate yield.[65] Pyrimidines **163d** with a free 2-amino group were cyclized to 5-amino-7-chlorooxazolo[5,4-*d*]pyrimidines **164d** in moderate to very good yields.[66]



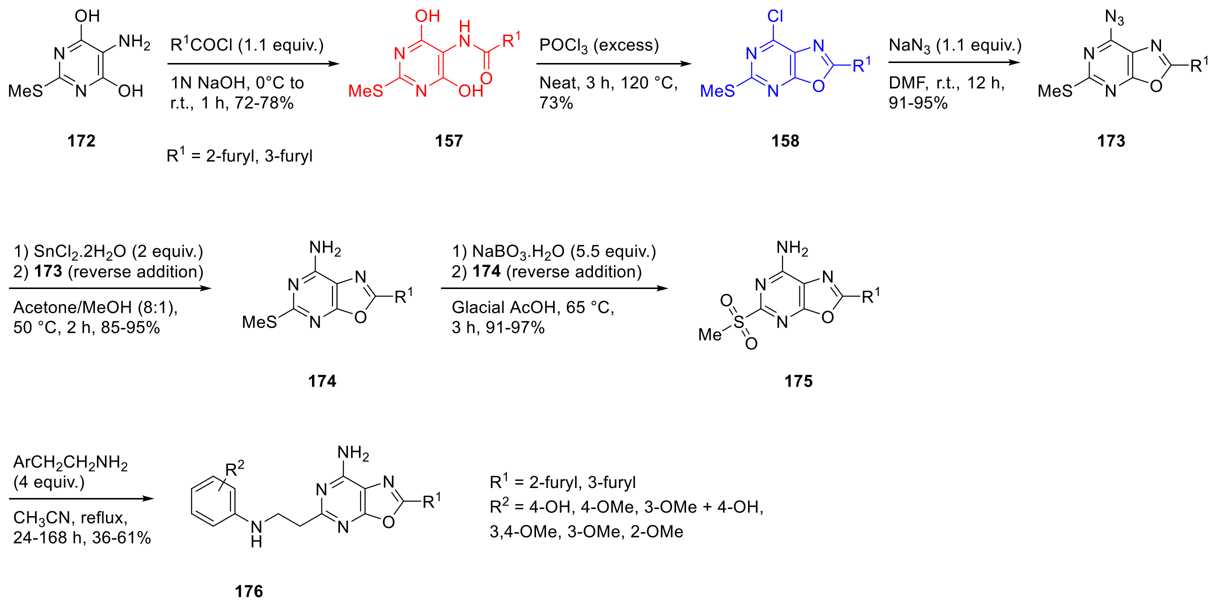
Scheme 27. Cyclization of 5-acylamino-4-hydroxypyrimidines utilizing excess N,N-dimethylaniline in phosphorus oxychloride

Pyrimidine **151**, lacking a free amino group, was cyclized to 7-chlorooxazolo[5,4-*d*]pyrimidine **152** in boiling phosphorus oxychloride in good yields (Scheme 28). Subsequent reaction with sodium thiosulfide in ethanol afforded the oxazolopyrimidine-7-thiones **165** in very good yields.[67] Pyrimidine **166** was cyclized to **167** in very good yield under similar reaction conditions. Subsequent substitution of the chlorine by ammonia afforded 7-aminooxazolopyrimidine **168**.[65] Remarkably, when dihydroxypyrimidine **169** was cyclized with phosphorus pentachloride, oxazolopyrimidine-7(6*H*)-one **170** was obtained. The isolated yield was not mentioned so it is not clear to what extent the oxygen at the 7-position was substituted by a chlorine atom, if at all. Further alkylation of this compound with 1-bromopropane afforded 7-propoxy derivative **171** in 18% yield and the 6-*N*-propyl regioisomer in 19% yield.[68]



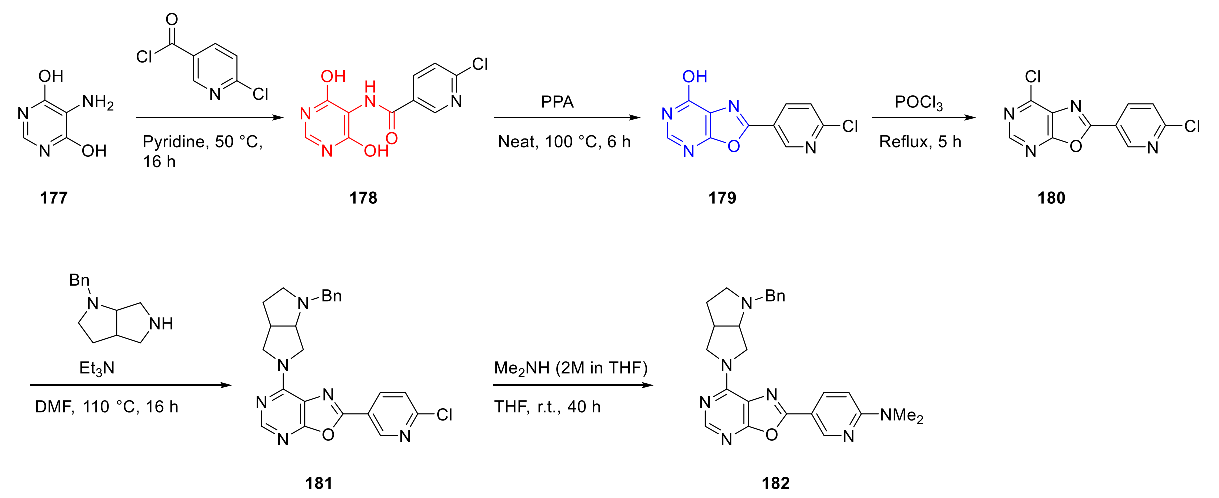
Scheme 28. Cyclization of 5-acylamino-4-hydroxypyrimidines utilizing phosphorus oxychloride or phosphorus pentachloride

Reaction of pyrimidine **172** with furoyl chlorides afforded the 4-acylaminopyrimidines **157** in good yields (Scheme 29). Subsequent ring closure with phosphorus oxychloride gave 7-chlorooxazolopyrimidine **158**, which then reacted with sodium azide to get 7-azidooxazolopyrimidine **173** in excellent yields. Reduction of the azide followed by oxidation of the sulfide afforded sulfone **175**, which was then reacted with various amines to give the final 2,5-disubstituted-7-aminooxazolopyrimidine **176**.[69]



Scheme 29. Application of the phosphorus oxychloride induced cyclization of 5-acylamino-4-hydroxypyrimidines

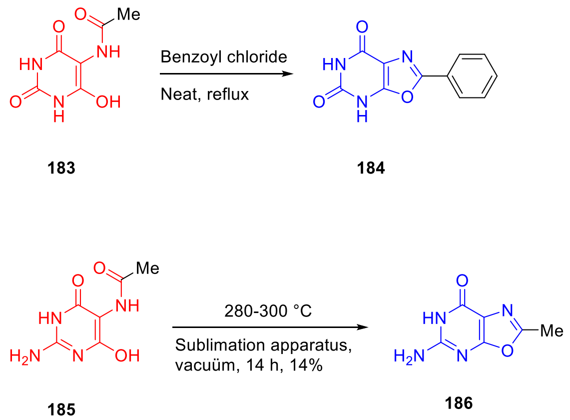
In a similar example, polyphosphoric acid (PPA) was used to ring close pyrimidine **178** to oxazolopyrimidine **179** (Scheme 30). The oxazolopyrimidine-7-ol **179** was subsequently chlorinated by phosphorus oxychloride to obtain 7-chlorooxazolopyrimidine **180**. Remarkably, the chlorine at the 7-position was substituted by a bipyrrolidine moiety in DMF at high temperature, while the chlorine on the pyridine ring was substituted in the next step by excess dimethylamine in THF at room temperature to yield compound **182**.[70]



Scheme 30. Cyclization of 5-acylamino-4-hydroxypyrimidines utilizing polyphosphoric acid

Besides phosphorus oxychloride or polyphosphoric acid, an acyl chloride such as benzoyl chloride can also effectuate ring closure. Refluxing acetyluramil **183** in benzoyl chloride delivered 2-phenyloxazolo[5,4-*d*]pyrimidine-5,7-dione **184** (Scheme 31). The presence of the phenyl group indicates that transacylation must have occurred. The acetyl group on acetyluramil is not very stable, and hydrolytic cleavage followed by benzoylation probably preceded the cyclization reaction.[71]

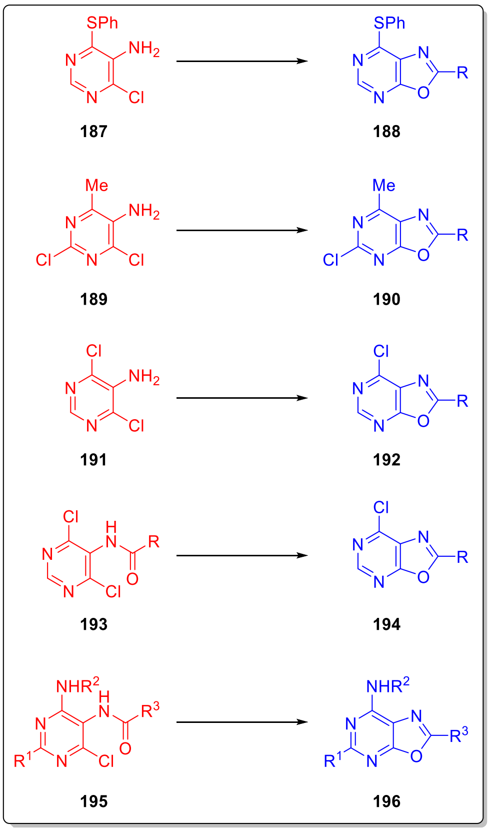
Ring closure was also established thermally, without the use of any reagent. Pyrimidine **185**, with an acetylated amino group at the 5-position and a hydroxyl group at the 4- and 6-positions, was ring closed without any condensing agent by making use of a sublimation apparatus at high temperature. The desired 5-amino-2-methyl-6*H*-oxazolo[5,4-*d*]pyrimidin-7-one **186** (8-methyl-9-oxoguanine) was obtained in very low yield.[72]



Scheme 31. Cyclization of 5-acylamino-4-hydroxypyrimidines under forcing conditions

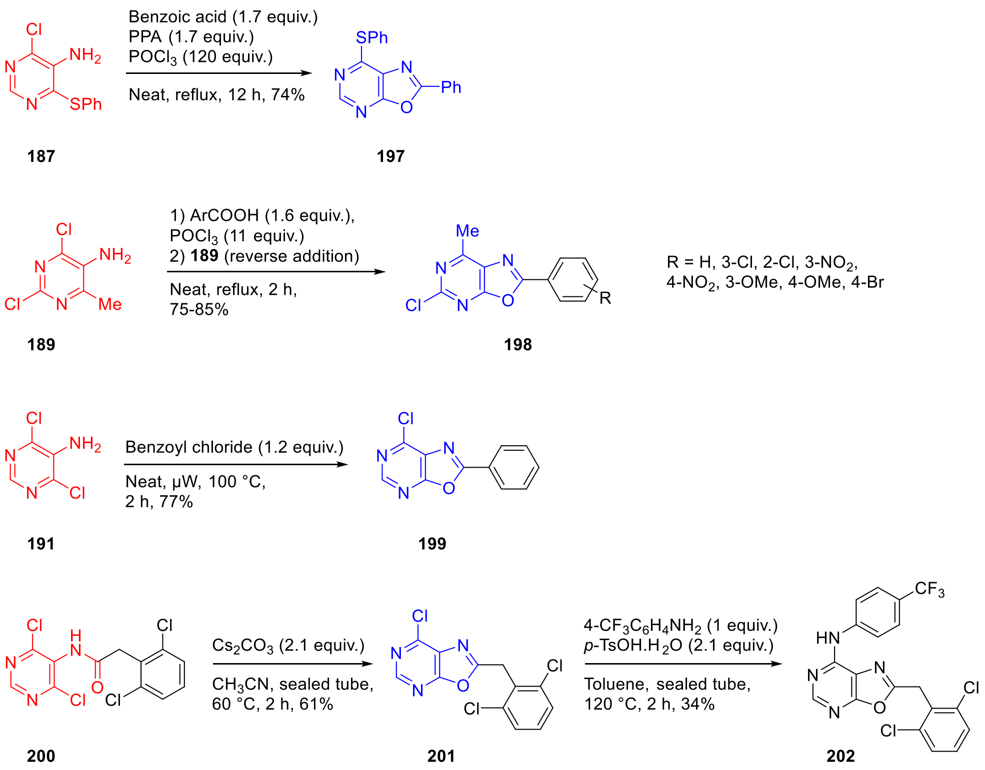
4.1.1.3 Starting from a 5-(acyl)amino-4(,6)-(di)chloropyrimidine

Another suitable building block is a 5-aminopyrimidine with a chlorine atom on the 4- and/or 6-positions. The amino group can either be free or acylated (Scheme 32).



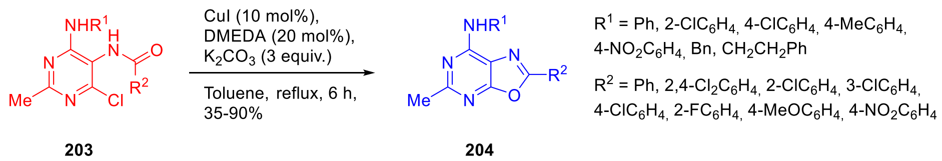
Scheme 32. Oxazolo[5,4-d]pyrimidines by annulation of oxazole rings to 5-amino-4-chloropyrimidines

Treatment of 4-chloro-5-aminopyrimidine **187** with equimolar amounts of benzoic acid and PPA in phosphorus oxychloride under reflux conditions afforded oxazolopyrimidine **197** in good yield (Scheme 33).[73] Similarly, treatment of 5-amino-2,4-dichloropyrimidine **189** with arylcarboxylic acids in refluxing $POCl_3$ gave 5-chlorooxazolo[5,4-*d*]pyrimidines **198** in very good yields.[74] 5-Amino-4,6-dichloropyrimidine **191** was treated with benzoyl chloride to obtain 7-chlorooxazolo[5,4-*d*]pyrimidine **199** in good yield.[75] Cyclization of acylated 5-amino-4,6-dichloropyrimidine **200** did not require phosphorus oxychloride or polyphosphoric acid and treatment with a simple inorganic base such as cesium carbonate in acetonitrile was sufficient to get the 7-chlorooxazolo[5,4-*d*]pyrimidine **201** in acceptable yield. Subsequent substitution of the chlorine with an appropriate amine afforded the 2,7-disubstituted oxazolopyrimidine **202**.[76]



Scheme 33. *Cyclizations of 5-acylamino-4-chloropyrimidines under forcing conditions*

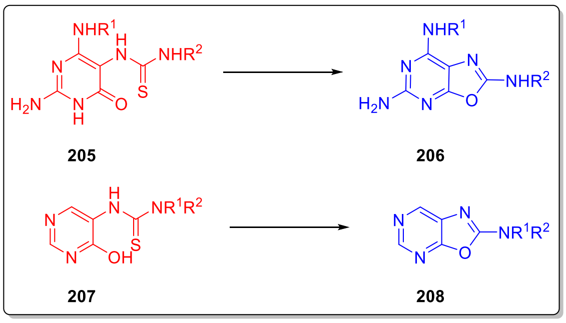
A facile synthesis of 2,5,7-trisubstituted oxazolo[5,4-*d*]pyrimidines **204** was developed by Xu *et al.* (Scheme 34). A combination of copper (I) and dimethylethylenediamine as ligand was used to catalyze the intramolecular C-O cross-coupling of the ortho-halopyrimidine amides **203** to provide the oxazolopyrimidines **204** in moderate to very good yields.[77]



Scheme 34. *Cyclization of 5-acylamino-4-hydroxypyrimidines by means of copper catalysis*

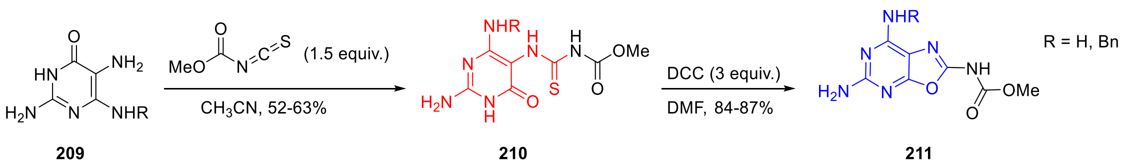
4.1.1.4 Cyclization of a 4-hydroxy-5-thioureidopyrimidine

Analogous to the 5-acylamino-4-hydroxypyrimidines, 4-hydroxy-5-thioureidopyrimidines can be cyclized to oxazolo[5,4-*d*]pyrimidines (Scheme 35).



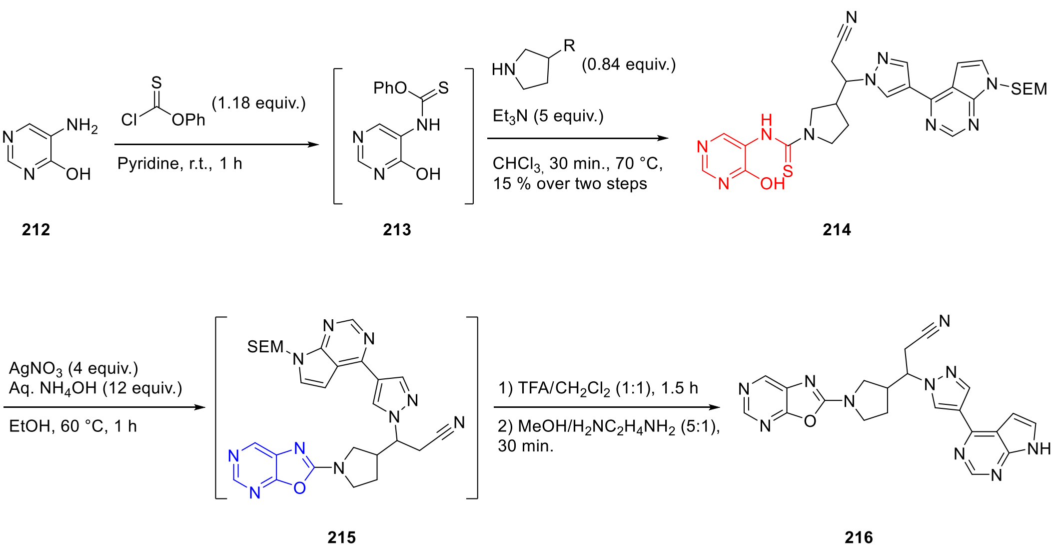
Scheme 35. Oxazolo[5,4-d]pyrimidines by cyclization of 4-hydroxy-5-thioureidopyrimidines

Pyrimidinone **209** was first reacted with methoxycarbonyl isothiocyanate to afford the thioureido intermediate **210** in moderate yield (Scheme 36). Ring closure was then effectuated by treatment with dicyclohexylcarbodiimide to get oxazolopyrimidines **211** in very good yields.[78]



Scheme 36. DCC induced cyclization of 4-hydroxy-5-thioureidopyrimidines

When 5-aminopyrimidin-4-ol **212** was treated with *O*-phenyl chlorothionoformate, an intermediate *O*-thiocarbamate **213** was formed (Scheme 37). This crude product was then reacted with a highly functionalized pyrrolidine to get thioureidopyrimidine structure **214** in very low yield. The oxazole ring cyclization was effectuated by treatment with silver nitrate and aqueous ammonium hydroxide in ethanol to afford the intermediate 2-substituted oxazolo[5,4-*d*]pyrimidine **215**. This crude product was then deprotected by stirring in a mixture of trifluoroacetic acid and dichloromethane, followed by stirring in a mixture of ethylenediamine and methanol to obtain **216**.[79]



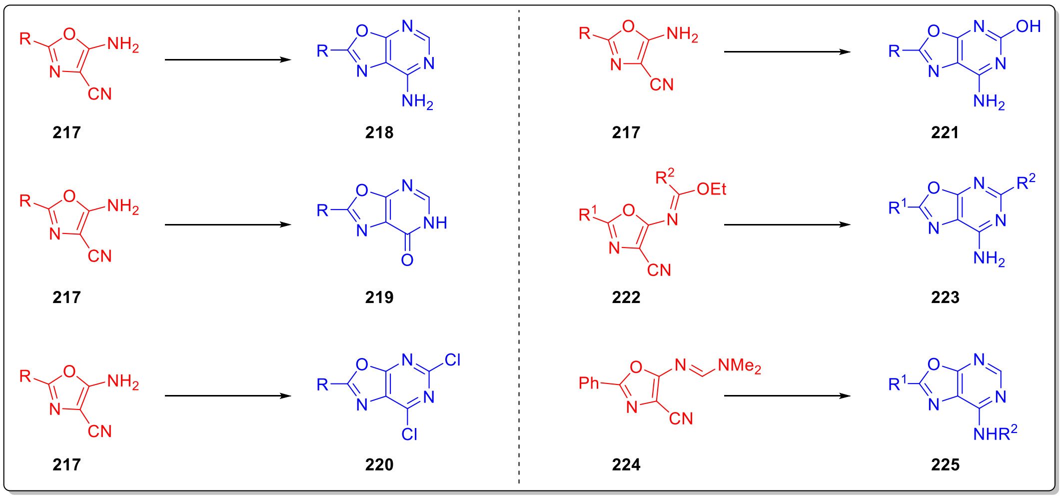
Scheme 37. Silver nitrate induced cyclization of 4-hydroxy-5-thioureidopyrimidines

4.1.2 Scaffold construction starting from a functionalized oxazole

A second synthetic pathway towards oxazolo[5,4-*d*]pyrimidines is the annulation of a pyrimidine ring on a functionalized oxazole. The oxazole usually has a free amino group at the 5-position and a nitrile, ester or amide group at the 4-position.

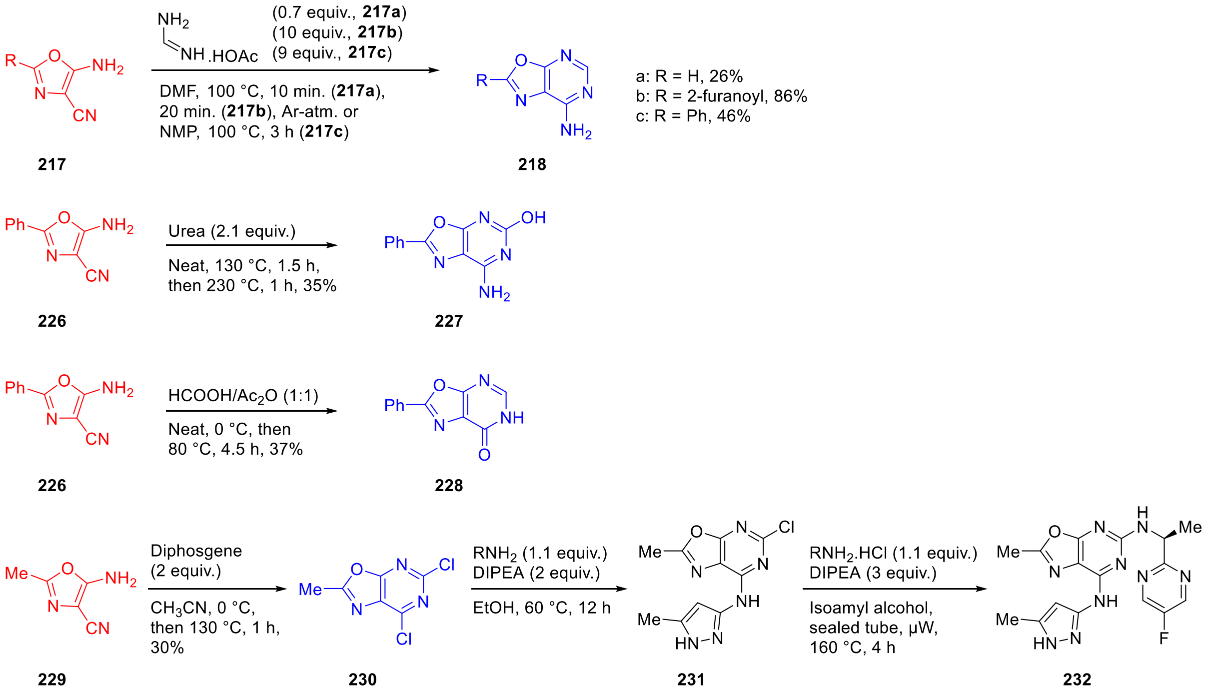
4.1.2.1 Starting from a 5-aminooxazole-4-carbonitrile

A C(2)-functionalized 5-aminooxazole-4-carbonitrile is a versatile building block for the construction of different oxazolo[5,4-*d*]pyrimidines (Scheme 38).



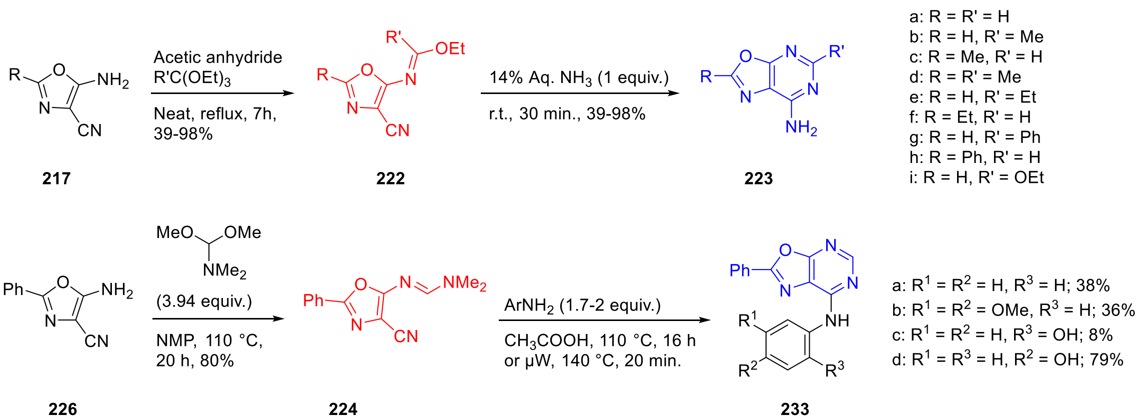
Scheme 38. Oxazolo[5,4-d]pyrimidines by annulation of pyrimidine rings to 5-aminooxazole-4-carbonitriles

Reaction of 4-cyano-5-aminooxazole **217a** with formamidine acetate delivered 7-aminooxazolo[5,4-*d*]pyrimidine **218a** (Scheme 39). The low yield was attributed to facile ring opening and degradation of the 5-amino-4-cyanooxazole.[80] Substitution of the 2-position of the oxazole with a 2-furanoyl or phenyl substituent improved the stability and accordingly products **218b-c** were obtained in higher yields.[65,81] Reaction of **226** with urea at high temperature gave 7-aminooxazolopyrimidin-5-ol **227**, while treatment with a mixture of formic acid and acetic anhydride at 80 °C afforded oxazolopyrimidin-7-one **228.**[81] Diphosgene was also applied in the direct ring closure of 5-aminooxazole-4-carbonitrile **229**, affording the 5,7-dichlorooxazolopyrimidine **230** in low yield. Subsequent substitution of the 7- and 5-position with appropriate amines resulted in compound **232.**[82]



Scheme 39. One-step condensation of 4-cyano-5-aminooxazoles with a variety of one-carbon synthons

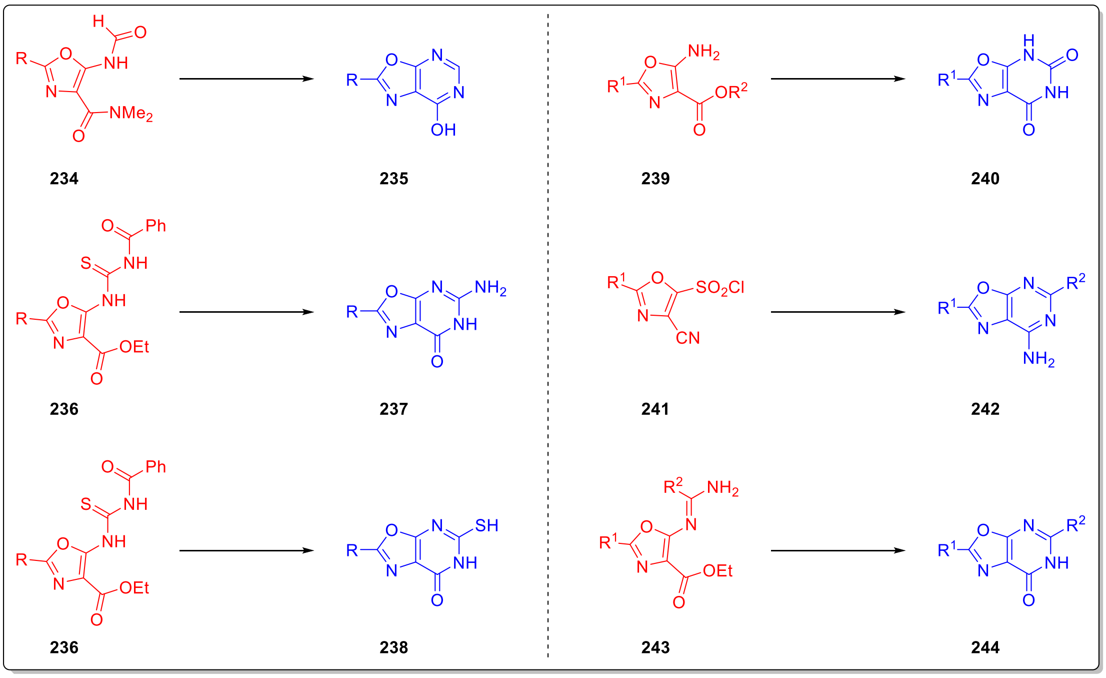
Pyrimidine ring annulation was also achieved in two steps. Reaction of oxazole **217** with an orthoester in refluxing acetic anhydride afforded the intermediate *N*-ethoxymethylene derivatives **222** in moderate to excellent yields (Scheme 40). Ring closure with aqueous ammonia then afforded the desired 7-aminooxazolo[5,4-*d*]pyrimidines **223**.[83] Instead of aqueous ammonia, aqueous solutions of various primary amines in water could also be used to obtain a substituted 7-amino group on this scaffold.[84] Reaction of **226** with *N*,*N*-dimethylformamide dimethyl acetal in NMP resulted in amidine **224** in very good yield. Subsequent cyclization by treatment with various anilines in acetic acid gave N-substituted oxazolopyrimidin-7-amines **233** in varying yields.[81]



Scheme 40. Two-step condensation of 4-cyano-5-aminooxazoles with acetic anhydride or N,N-dimethylformamide dimethyl acetal

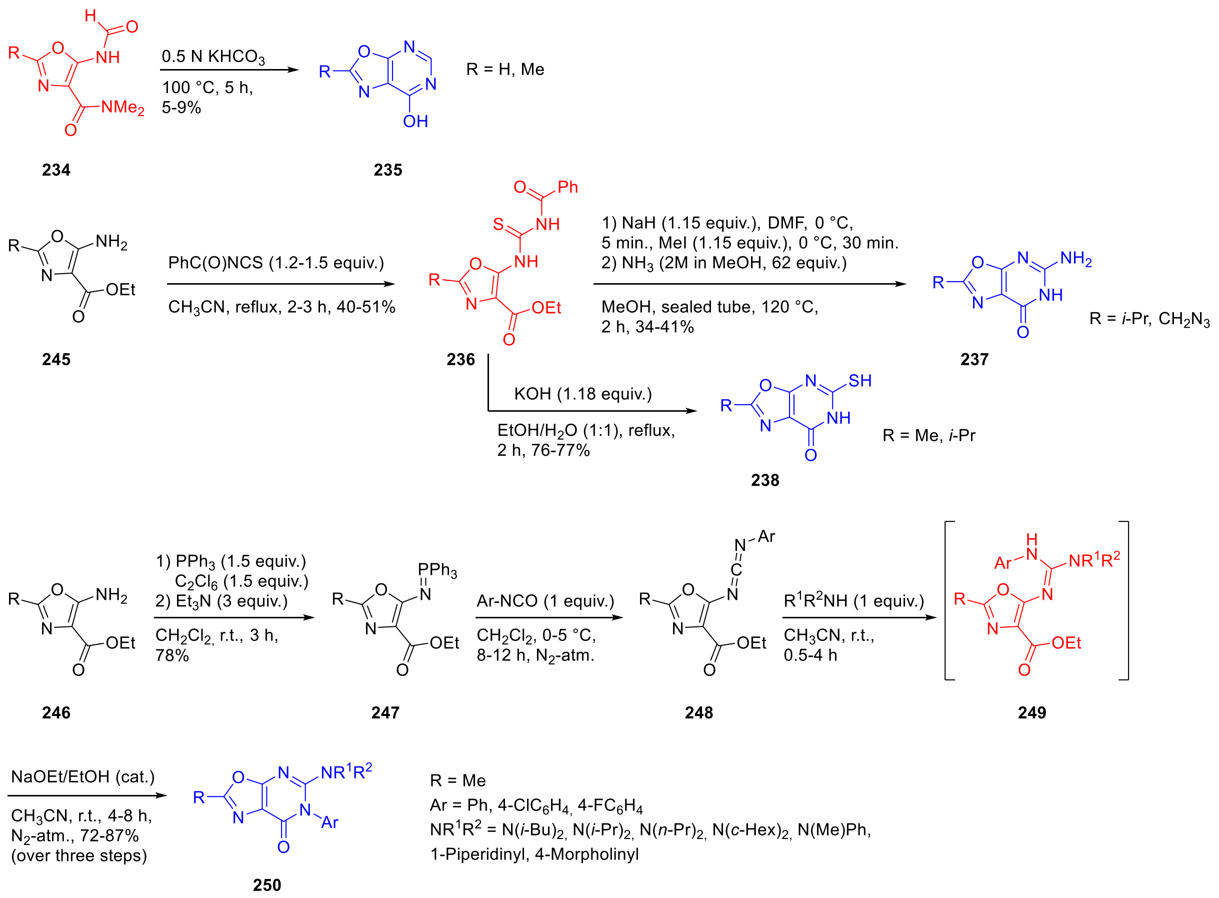
4.1.2.2 Starting from various other 2,4,5-trisubstituted oxazoles

A variety of other 2,4,5-trisubstituted oxazoles were applied as building blocks for the construction of oxazolo[5,4-*d*]pyrimidines (Scheme 41).



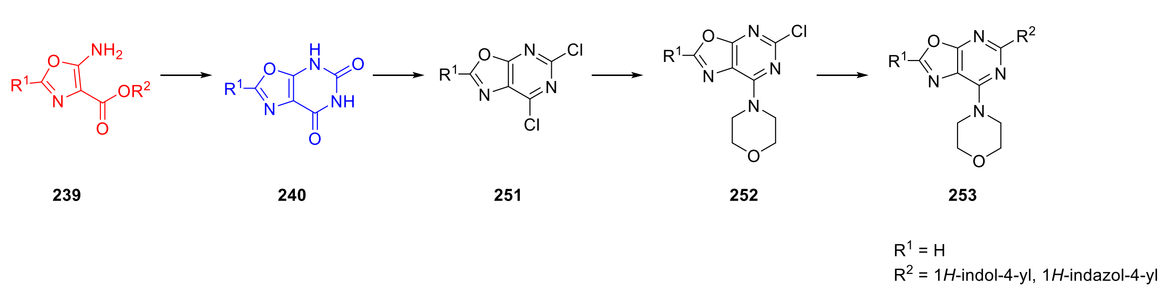
Scheme 41. *Oxazolo[5,4-d]pyrimidines by annulation of pyrimidine rings to oxazoles with appropriate substituents at positions 4 and 5*

Formylated 5-aminooxazole **234** was cyclized to oxazolo[5,4-*d*]pyrimidine-7-ols **235** in very low yield by treatment with a boiling potassium bicarbonate solution (Scheme 42).[85] When ethyl 5-aminooxazole-4-carboxylate **245** was treated with benzoyl isothiocyanate, thiourea **236** was obtained in moderate yields. Direct cyclization of these thiourea structures towards the 5-aminooxazolo[5,4-*d*]pyrimidin-7(6*H*)-ones **237** with ammonia in methanol was unsuccessful. This cyclization was achieved in low yields by a step-wise methylation, followed by cyclization in the presence of ammonia in methanol. Alternatively, thiourea **236** was ring closed to 5-mercaptooxazolo[5,4-*d*]pyrimidin-7(6H)-one **238** by aqueous potassium hydroxide in ethanol in good yields.[86] Ethyl 5-aminooxazole-4-carboxylate **246** was also used to construct oxazolo[5,4-*d*]pyrimidinones **250** through a consecutive aza-Wittig reaction. Treatment with triphenylphosphine, hexachloroethane and triethylamine produced iminophosphorane **247** in good yield. Further reaction with isocyanates afforded the carbodiimide intermediates **248**, which reacted smoothly with secondary amines to generate guanidines **249**. Ring closure to the desired oxazolopyrimidinones **250** was effectuated by a catalytic amount of sodium ethoxide in good overall yields.[87]



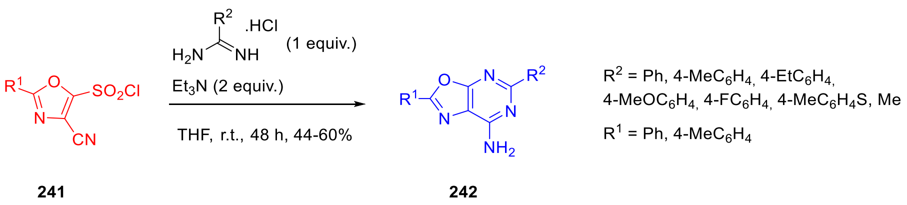
Scheme 42. Annulation of pyrimidine rings to 4-carboxy-5-aminooxazoles using a variety of one-carbon synthons

In the same study on protein kinase B (PKB) inhibitors as mentioned in section 3.1.2 on oxazole[4,5-*d*]pyrimidines, two new C(2)/C(4)-functionalized oxazolo[5,4-*d*]pyrimidines **253** were synthesized (Scheme 43). Unfortunately, the synthesis and characterization of these compounds was not described in detail. The only structures of Scheme 43 which were unambiguously reported are the final structures **253**. According to a general reaction scheme, the starting material was 5-aminooxazole **239** with an ester group at the 4-position and optionally a substituent at the 2-position. This oxazole was cyclized to afford oxazolo[5,4-*d*]pyrimidin-5,7(4*H*,6*H*)-dione **240**. Chlorination of this compound then lead to 5,7-dichloropyrimidine **251**, which was subsequently substituted at the 7- and 5-position to obtain the final structures **253**. No biological results were reported.[56]



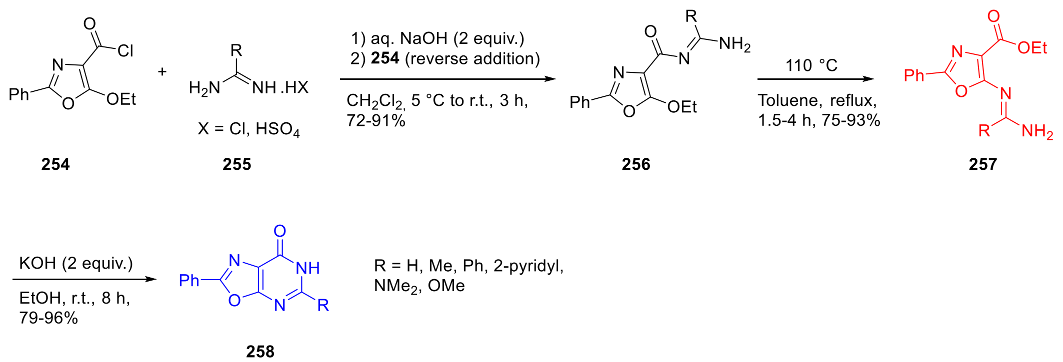
Scheme 43. Application of the annulation of pyrimidine rings to 4-carboxy-5-aminooxazoles

All of the aforementioned reactions require heating of the reaction mixture, which may be undesirable when fragile substituents are involved. Therefore, Kornienko and coworkers developed a method to construct functionalized oxazolopyrimidines under mild reaction conditions. Reacting 4-cyanooxazole-5-sulfonyl chlorides **241** with various amidines at room temperature in THF afforded the desired oxazolopyrimidines **242** in moderate yields (Scheme 44).[88,89]



Scheme 44. Annulation of pyrimidine rings to 4-cyanooxazole-5-sulfonyl chlorides

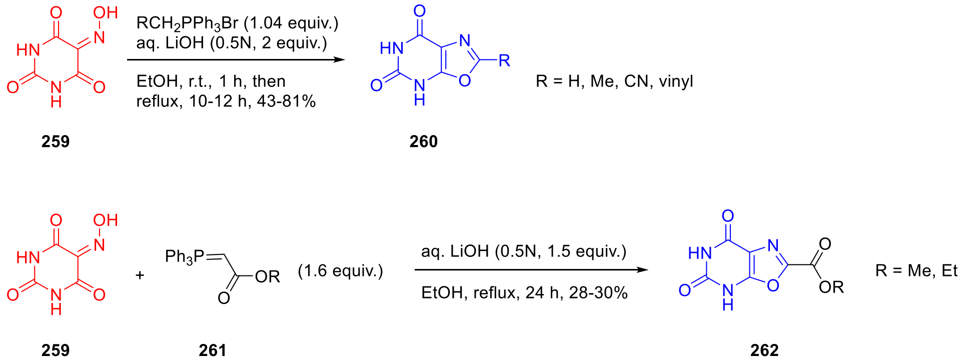
The Cornforth rearrangement is a reaction which is specific for 4-acyloxazoles. This rearrangement was used to develop a new synthetic pathway towards oxazolo[5,4-*d*]pyrimidin-7-ones **258** (Scheme 45). Reaction of 4-acyloxazole **254** with an appropriate amidine **255** delivered the intermediate acylamidines **256** in good yields. Thermolysis in refluxing toluene induced the Cornforth rearrangement reaction and gave the amidines **257** in good to excellent yields. These were subsequently cyclized to oxazolopyrimidin-7-ones **258** by treatment with ethanolic potassium hydroxide in very good yields.[90]



Scheme 45. Synthesis of oxazolo[5,4-d]pyrimidin-7-ones through the Cornforth rearrangement

4.1.3 Scaffold construction starting from violuric acid

Violuric acid (**259**) can be converted to 2-substituted oxazolo[5,4-*d*]pyrimidine-5,7(4*H*,6*H*)-diones by reaction with triphenylphosphonium salts or triphenylphosphonium ylides (Scheme 46). Thus, treating violuric acid with various triphenylphosphonium bromides and aqueous lithium hydroxide in ethanol afforded the oxazolopyrimidine-5,7-diones **260** in moderate to good yields. No reaction occurred when ester triphenylphosphonium bromides were used, while the corresponding ester ylides **261** delivered the 2-carboxylated oxazolopyrimidinones **262** in low yields.[91]



Scheme 46. Synthesis of oxazolo[5,4-d]pyrimidine-5,7(4H,6H)-diones through the condensation of violuric acid and Wittig reagents

4.2 Biological activity

4.2.1 Receptor modulators

Compound **142** (Figure 9, Scheme 25) is an *in vitro* $P2Y_1$ receptor antagonist with a K_i value of 24 nM. The $P2Y_1$ receptor is an important receptor for ADP in platelets. In turn, platelet activation is known to play a pivotal role in thrombus formation. Therefore, $P2Y_1$ antagonists could have significant utility in the treatment of a variety of thrombotic disorders. Compound **142** inhibits ADP-induced platelet aggregation with an IC_{50} value of 3.9 µM.[92]

Compound **168** (Figure 9, Scheme 28) is an A_2 adenosine receptor antagonist with an IC_{50} value below 100 nM. This receptor plays an important role in the regulation of glutamate and dopamine release, and could be useful in treatment of conditions such as pain, insomnia, depression, drug addiction and Parkinson's disease.[65] **168** was used as a lead compound in a later study on the development of selective A_{2A} adenosine receptor antagonists for positron emission tomography (PET). This resulted in compounds **176** (Scheme 29). Compounds **176** are antagonists of the A_{2A} adenosine receptor ($A_{2A}AR$) with IC_{50} values ranging from 14-33 nM and of the A_1 adenosine receptor (A_1AR) with IC_{50} values ranging from 170-1700 nM, resulting in A_{2A}/A_1 selectivity ratios ranging from 5 to 91. However, a high degree of unspecific binding was observed *in vitro*, rendering these compounds useless as PET ligands.[69]

Compound **263** (Figure 9) is an angiotensin II receptor antagonist with an IC_{50} value of 98 nM. This receptor belongs to the class of GPCRs and is responsible for the signal transduction of the vasoconstricting stimulus of the peptide hormone angiotensin II. Antagonists of this receptor can be useful in the control of hypertension and related illnesses.[93]

Compound **202** (Figure 9, Scheme 33) is an *in vitro* antagonist of the human transient receptor potential protein vanilloid receptor 1 (TRPV1) with an IC_{50} value of 136 nM. TRPV1 antagonists may be useful in disorders or conditions mediated by TRPV1 activity, such as pain, itch, arthritis, cough, asthma or inflammatory bowel disease.[76]

Compound **264** (Figure 9, Scheme 34) is an inhibitor of the vascular endothelial growth factor receptor 2 (VEGFR-2) with an IC_{50} value of 0.33 µM. The vascular endothelial growth factor is a key mediator of angiogenesis, which is a critical process in solid tumor progression. Compound **264** possesses antiproliferative activity against HepG2 and U251 cells with IC_{50} values of 15.5 and 15.3 µM, respectively, and was active against VEGF-induced human umbilical vein endothelial cell (HUVEC) proliferation with an IC_{50} value of 0.29 µM.[94] Compound **265** (Figure 9) is an inhibitor of VEGFR-2 with an IC_{50} value of 0.3 µM. However, it was not selective and also inhibits EGFR with an IC_{50} value of 0.2 µM.[95]

Compound **266** (Figure 9) is an agonist of the endothelial differentiation gene receptor 1 (EDG-1) and was three times more active than the positive control substance sphingosine 1-phosphate at 10 µM.[68] Further work lead to compound **267**, an EDG-1 agonist which effectively reduced the severity of acute kidney disease (AKI) in a murine *in vivo* model.[96]

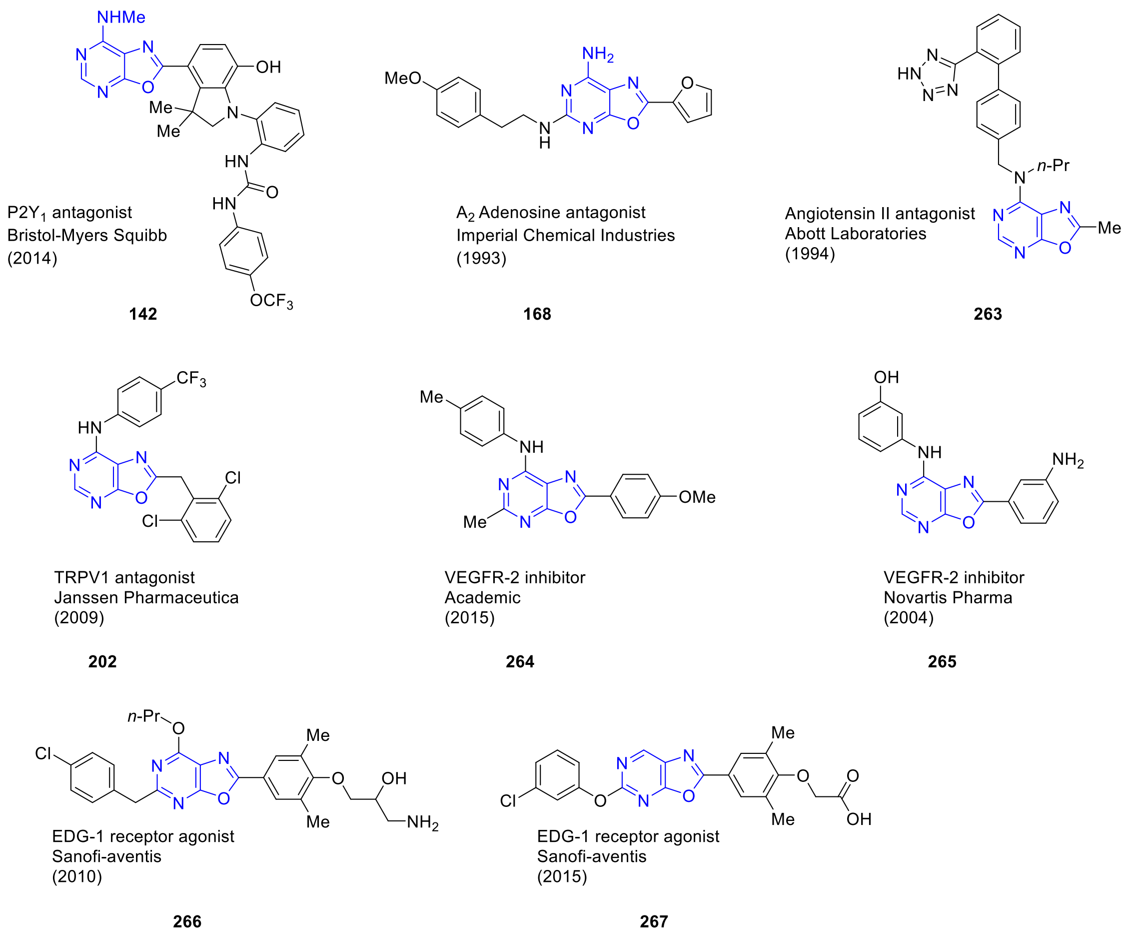


Figure 9. Oxazolo[5,4-d]pyrimidines reported as receptor modulators

4.2.2 Kinase inhibitors

The 2,7-disubstituted oxazolo[5,4-*d*]pyrimidine **182** (Figure 10, Scheme 30) is an inhibitor of adenosine kinase (AK) with an IC_{50} value of 10 nM. AK inhibition is an attractive therapeutic approach for several conditions such as neurodegeneration, seizures, ischemia, pain and inflammation.[70] Compounds **268a-b** inhibit AK with K_i values of 11.1 and 25.2 µM, respectively. The latter also showed selectivity for adenosine A_1 over A_{2A} receptors.[97]

Compounds **269** (Figure 10) are *in vitro* inhibitors of Aurora A kinase with IC_{50} values between 1 and 50 nM. Aurora kinases belong to the serine/threonine subclass of kinases and are involved in the regulation of mitosis. The derivative with a 4-pyridyl substituent at the 2-position was active against HCT-116 cancer cells *in vitro* with a cytotoxicity IC_{50} value lower than 100 nM.[98]

Compound **216** (Figure 10, Scheme 37) is a JAK1 inhibitor with an IC_{50} value between 50 and 100 nM. JAK1 is a tyrosine kinase and plays an important role in initiating responses to cytokine receptors. Expression of JAK1 in cancer cells is related to metastasis.[79] Compound **232** (Figure 10, Scheme 39) is an *in vitro* inhibitor of JAK2 kinase with an IC_{50} value of 2.3 µM. Remarkably, its thiazolo[5,4-*d*]pyrimidine analogue, with a sulfur atom in structure **232** instead of an oxygen atom, has an IC_{50} value of 3 nM.[82]

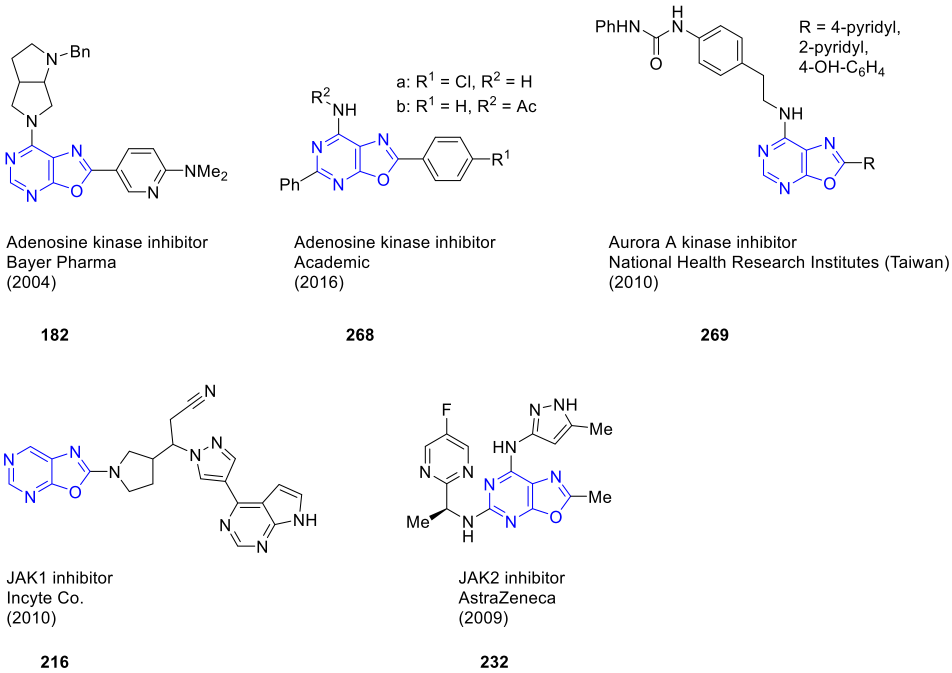


Figure 10. Oxazolo[5,4-d]pyrimidines reported as kinase inhibitors

4.2.3 Miscellaneous biological activity

Compound **186** (Figure 11, Scheme 31) is an effective inhibitor of ricin toxin A chain (RTA) with an IC_{50} value of 0.40 mM.[72] It was also found to be an effective inhibitor of Shiga toxin 1 A1 (Stx1A1) with an IC_{50} value of 2 mM.[99] Ricin is a highly toxic lectin (a carbohydrate-binding protein) which naturally occurs in the seeds of the castor oil plant, *Ricinus communis*. The Shiga toxin is produced by *Shigella dysenteriae* and shigatoxigenic serotypes of *Escherichia coli*. The structurally similar purine isosteres **270** are competitive inhibitors of human erythrocytic hypoxanthine-guanine phosphoribosyl transferase (HGPRT) with K_i values ranging from 84 to 173 µM. HGPRT plays a central role in the generation of purine nucleotides through the purine salvage pathway. Inhibitors of HGPRT could be useful to treat schistosomiasis and malaria, diseases in which the parasites lack the *de novo* purine biosynthetic pathway.[100]

Nucleoside analogues 6-(β-D-ribofuranosyl)oxazolo[5,4-*d*]pyrimidin-7-ones **271** (Figure 11) were prepared by a standard glycosylation reaction on trimethylsilylated 2-substituted oxazolo[5,4-*d*]pyrimidin-7-ones. The less bulky nucleoside analogues (R = Me, Et, *n*-Pr) inhibit the proliferation of leukemia L1210 cells with IC_{50} values between 7 and 50 µM while the more bulky (R = *i*-Pr, Ph) derivatives had IC_{50} values higher than 100 µM. They also inhibit the proliferation of E. coli K_{12} cells with IC_{50} values between 0.5 and 6 µM for the *n*-propyl and ethyl group respectively, while for methyl and the bulky substituents, IC_{50} values higher than 100 µM were observed.[101]

Compound **272** (Figure 11) is an inhibitor of the NEDD8-activating enzyme (NAE) with an IC_{50} value below 500 nM. Activated NEDD8 is needed in two DNA repair pathways. NAE inhibition may cause deficient DNA repair leading to accumulation of DNA damages, eventually resulting in cell death. Cancer cells may be more sensitive to this effect compared to normal cells if the cancer cells are independently deficient in DNA repair.[75]

Compound **273** (Figure 11) is an activator of the caspase cascade in different cancer cell lines with EC_{50} values between 58-93 nM. Caspases are a family of protease enzymes that play essential roles in programmed cell death and inflammation.[59]

Compound **274** (Figure 11) is an *in vitro* inhibitor of plasma kallikrein (PK) with an IC_{50} value of 1.78 µM. PK is the activated form of the trypsin-like serine protease plasma-prokalliokrein and is mainly expressed by hepatocytes in the liver. PK inhibitors are considered to be useful in the treatment of a wide range of disorders, in particular retinopathy or edema-associated diseases, such as hereditary angioedema, macular edema and brain edema.[102]

Compound **275** (Figure 11) inhibits acetyl-CoA carboxylase 2 (ACC2) *in vitro* with an IC_{50} value of 0.34 µM. ACC2 is one of the two isoforms of ACC in mammals, and regulates the metabolism of fatty acids. Inhibitors of ACC2 could be useful in the treatment of obesity and diabetes.[103]

Compound **276** (Figure 11) is an immunosuppressing agent and inhibits the *in vitro* Mixed Lymphocyte Reaction (MLR) test with an IC_{50} value of 3 µM.[104] This type of structures also possesses antiviral activity against poliovirus and hepatitis C genotype 1a (HCV-1a).[105]

Compound **277** (Figure 11) inhibits human umbilical vein endothelial cell (HUVEC) proliferation *in vitro* with an IC_{50} value of 9.3 µM and possesses superior anti-angiogenic activity.[106] Further research by the same group lead to a similar compound, **278**, which also possesses anti-angiogenic activity and inhibits HUVEC proliferation *in vitro* with an IC_{50} value of 12.4 µM. Despite the higher IC_{50} value, compound **278** had a better dose-response curve than compound **277** and was preferred for further study.[107]

Compound **279** (Figure 11) is an antiviral compound with activity against respiratory syncytial virus (RSV) and inhibits replication of the rgRSV224 virus *in vitro*.[108]

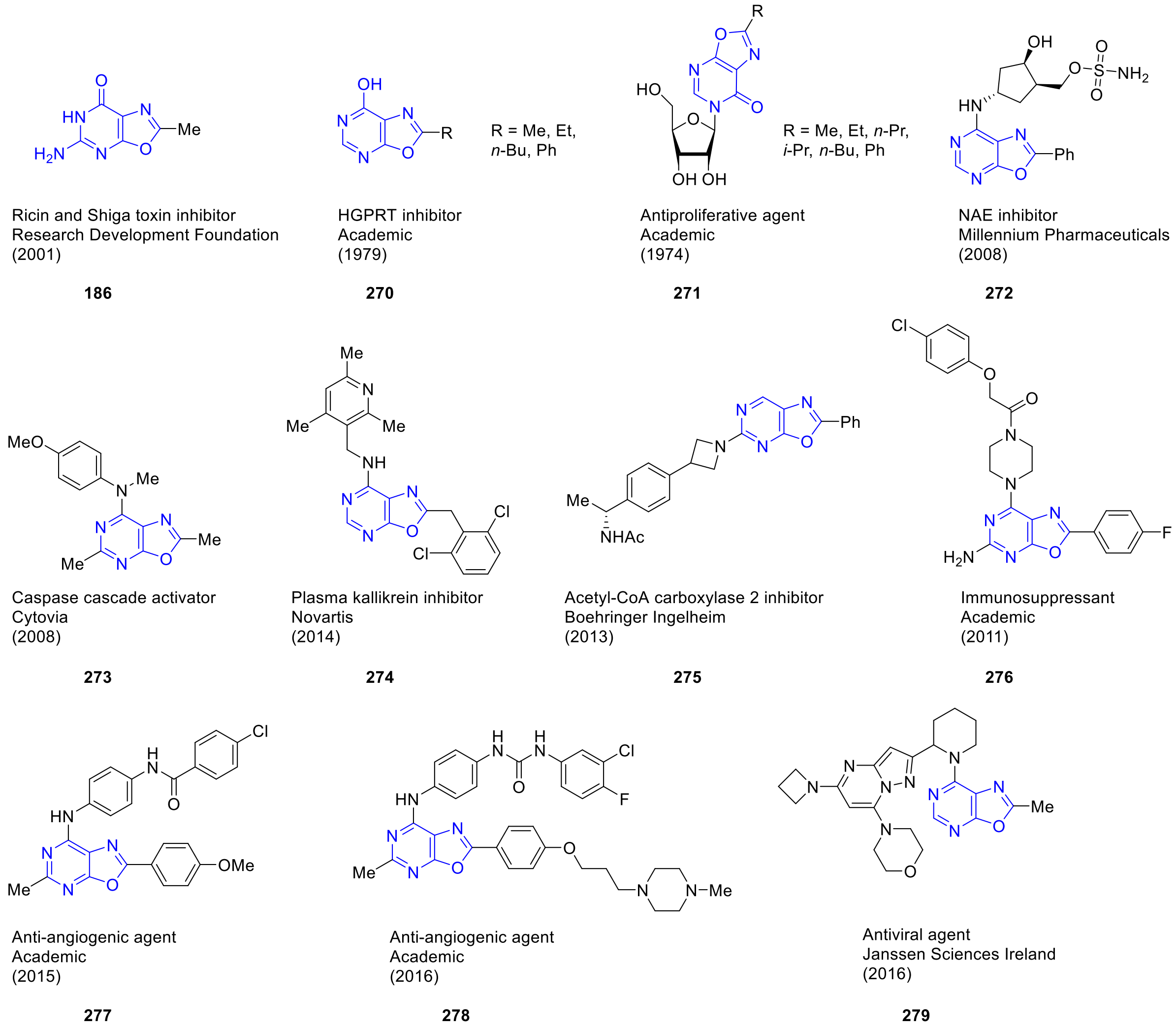


Figure 11. Oxazolo[5,4-d]pyrimidines of assorted biological interest

5 Conclusions

This literature overview demonstrates that the furo[3,4-*d*]pyrimidines are an underexplored class of compounds, both from a synthetic and medicinal point of view. Most likely, the lack of biological studies goes hand in hand with the paucity of synthetic methodologies to access this scaffold. Therefore, a window of opportunity exists to explore new synthetic avenues in the field of furo[3,4-*d*]pyrimidines.

Contrary to the furo[3,4-*d*]pyrimidines, the 5,7-dihydrofuro[3,4-*d*]pyrimidines (without substituents on the dihydrofuran ring) have been explored much more. Although the number of synthetic pathways is rather limited, synthetic access to a few key building blocks allowed this scaffold to be included in a variety of biological studies. This resulted in more than ten examples of bioactive compounds.

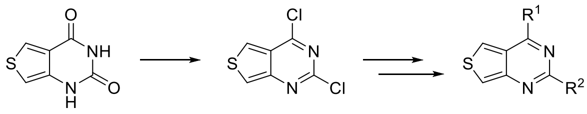
The oxazolo[4,5-*d*]pyrimidines again form a class of underexplored compounds, both from a synthetic and medicinal point of view, although less dramatically than the furo[3,4-*d*]pyrimidines. Once again, this probably results from the limited synthetic access to key building blocks for this type of scaffold. Therefore, there is still room for exploration in the field of oxazolo[4,5-*d*]pyrimidines.

Contrary to the oxazolo[4,5-*d*]pyrimidines, the oxazolo[5,4-*d*]pyrimidines are a well-explored class of compounds. A broad array of synthetic pathways is available to access oxazolo[5,4-*d*]pyrimidines with a variety of substitution patterns. This scaffold has been included in many biological studies, and this has resulted in more than twenty examples of bioactive compounds. Depending on the substitution pattern, various receptor (ant)agonists, kinase inhibitors, antiproliferative compounds and other inhibitors were obtained.

Results and discussion

1 Synthesis of furo[3,4-*d*]pyrimidine analogues

The synthetic strategy to gain access to furo[3,4-*d*]pyrimidines was based on a literature search of closely related structures. Thieno[3,4-*d*]pyrimidines were found to be the most similar compounds, and they are generally synthesized *via* the thieno[3,4-*d*]pyrimidine-2,4-dione **280** (Scheme 47). Chlorination of this precursor gives the 2,4-dichloropyrimidine **281**, which is then modified by replacement of the chlorine atoms by different substituents to give thieno[3,4-*d*]pyrimidines **282**.[109,110]



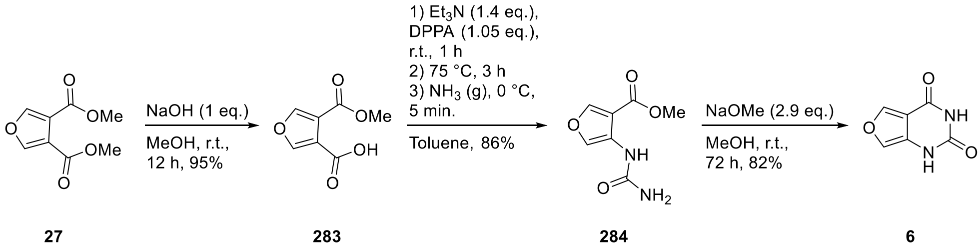
280 **281** **282**

Scheme 47. Synthetic pathway towards thieno[3,4-d]pyrimidines

2,4-Dichlorofuro[3,4-*d*]pyrimidine, the furan analogue of **281**, has not been described yet in the literature, but its precursor furo[3,4-*d*]pyrimidine-2,4-dione, which is the furan analogue of **280**, was already reported in two papers (Scheme 3, Scheme 4).[29,30] Neither of the two reported synthetic pathways gave straightforward, high-yield access to the desired building block. Therefore, a more convenient approach was highly desired.

1.1 Synthesis of the furo[3,4-*d*]pyrimidine-2,4-dione scaffold and subsequent reaction with phosphorus oxychloride

The two existing routes started from dimethyl/diethyl furan-3,4-dicarboxylate because it is commercially available and it has functional groups at the right positions. Therefore, a new synthetic route starting from the same furan dicarboxylate was the first consideration. The unselective aminolysis step was replaced by a selective partial hydrolysis with sodium hydroxide[111] and afforded the mono-acid **283** in 95% yield (Scheme 48). Then, the acid was converted to an intermediate isocyanate *via* a Curtius rearrangement by reaction of diphenyl phosphoryl azide and triethylamine as a base.[112] Ammonia gas was then bubbled through the reaction for five minutes at 0 °C to form the ureid **284** in 86% yield. The ureid could be ring-closed to the desired furo[3,4-*d*]pyrimidine-2,4-dione **6** in 82% yield by treatment with sodium methoxide in dry methanol.[113] Attempts to speed up this ring closure reaction by heating of the reaction mixture resulted in a significant increase in the amount of side products. Overall, the desired building block **6** was obtained in three reaction steps and 67% yield.



Scheme 48. Three-step synthesis of furo[3,4-d]pyrimidine-2,4-dione

The next step was the conversion of the pyrimidine-2,4-dione to the 2,4-dichloropyrimidine. In the literature, four procedures are reported to accomplish this conversion on thieno[3,4-*d*]pyrimidine-2,4-dione, all of them make use of phosphorus oxychloride as chlorinating reagent (Table 1).

Table 1. Reported methods to chlorinate the thieno[3,4-d]pyrimidine-2,4-dione scaffold

Reference	$POCl_3$ (mL/mmol **280**)	Base (mL/mmol **280**)	T (°C)	t (h)	Yield (%)
Okubo *et al.*[114]	1.7	*N*,*N*-Dimethylaniline (0.3)	100	2.5	43
Dong *et al.*[115]	0.8	*N*,*N*-Dimethylaniline (0.1)	105	16	81
Block *et al.*[110]	4.3	*N*,*N*-Diethylaniline (0.4)	105	16	92
Kim *et al.*[116]	3.4	/	106	3	39

These literature procedures were evaluated for the chlorination of furo[3,4-*d*]pyrimidine-2,4-dione. The reactions were followed by TLC analysis and in the case of a potentially successful reaction, *i.e.* conversion to a more apolar spot on TLC, the reaction was worked up and analyzed by LC-MS and NMR spectroscopy. The results are summarized in Table 2. The desired dichloropyrimidine was not detected in any of the reactions. Since the successful transformation of thieno[3,4-*d*]pyrimidine-2,4-dione and 5,7-dihydrofuro[3,4-*d*]pyrimidine-2,4-dione to their respective 2,4-dichloropyrimidine counterparts by treatment with phosphorus oxychloride has been described already, it can be concluded that the furan ring is responsible for the failure of this reaction. A possible explanation could be the relatively small aromatic stabilization in furan, which enables diene chemistry and enol ether chemistry, leading to the formation of bicyclic adducts, ring-opened adducts and polymeric material.

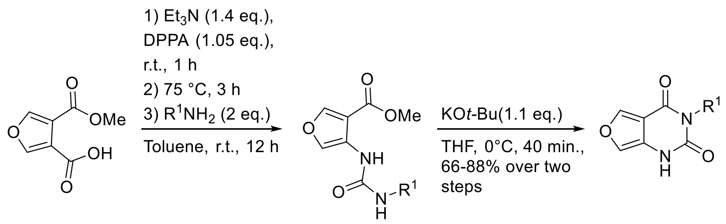
Table 2. Attempts to synthesize 2,4-dichlorofuro[3,4-d]pyrimidine using literature procedures

Procedure	Reaction time (h)	Results
Okubo *et al.*[114]	0.5	Starting material + apolar side products
	1	Full conversion to apolar side products
Dong *et al.*[115]	0.5	Starting material + apolar side products
	1	Full conversion to apolar side products
Dong *et al.*, at 23 °C	0.5	Starting material + apolar side products
	3	Starting material + apolar side products
Kim *et al.*[116]	0.5	Starting material + polar side products
	3	Starting material + polar side products

1.2 Synthesis of *N*-3 functionalized furo[3,4-*d*]pyrimidine-2,4-diones

Without the 2,4-dichlorofuro[3,4-*d*]pyrimidine building block, the predetermined route towards functionalized furo[3,4-*d*]pyrimidines was blocked. Therefore, other target structures were investigated for valorization of the developed synthetic methodology. First of all, *N*-1/*N*-3 functionalized furo[3,4-*d*]pyrimidine-2,4-diones appeared to be interesting structures. When the furo[3,4-*d*]pyrimidine-2,4-dione building block is treated with a base and an electrophile, the *N*-1 nitrogen will react selectively and *N*-1 functionalized furo[3,4-*d*]pyrimidine-2,4-diones will be obtained.[33] This impedes access to *N*-3 functionalized furo[3,4-*d*]pyrimidine-2,4-diones by demanding a *N*-1 protection-deprotection sequence. One synthetic pathway was reported already towards *N*-3 functionalized furo[3,4-*d*]pyrimidine-2,4-diones (Scheme 5). After six reaction steps, the overall yield was only 12-31%.[31]

To synthesize these structures in a faster and more convenient way, appropriate amines were added to the intermediate isocyanate of the Curtius rearrangement reaction to form the ureids **36** (Scheme 49). Most of the impurities were removed by silica gel column chromatography, and cyclization of the ureids **36** was effectuated by treatment with a base. The secondary ureids **36** were more susceptible towards ring closure than the primary ureid **284**, and the sodium methoxide-methanol system was replaced by potassium *tert*-butoxide in tetrahydrofuran at 0 °C for 40 minutes. The *N*-3 functionalized furo[3,4-*d*]pyrimidine-2,4-diones **38** were obtained in 66-88% yield over two steps (Table 3).[25]



283 **36** **38**

Scheme 49. Straightforward synthesis of N-3 functionalized furo[3,4-d]pyrimidine-2,4-diones

*Table 3. Structures and yields of compounds **38***

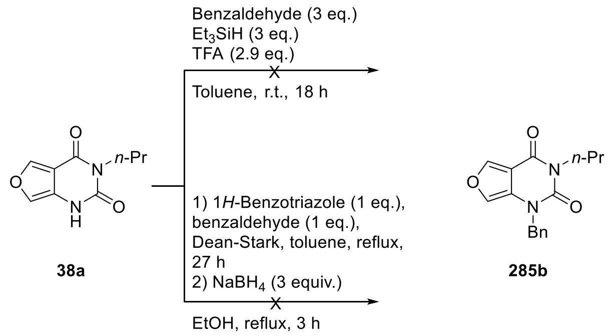
Compound	R	Yield (%)[a]
38a	*n*-Pr	88
38b	Bn	77
38c	2-Picolyl	66
38d	$4\text{-MeOC}_6H_4CH_2$	76
38e	$2\text{-MeOC}_6H_4CH_2$	79
38f	$3\text{-MeOC}_6H_4CH_2$	74
38g	$2\text{-FC}_6H_4CH_2$	75
38h	$3\text{-FC}_6H_4CH_2$	71
38i	$3,4,5\text{-(MeO)}_3C_6H_4CH_2$	81
38j	$2,3\text{-(MeO)}_2C_6H_4CH_2$	77
38k	$4\text{-CF}_3C_6H_4CH_2$	82
38l	$4\text{-PhOC}_6H_4CH_2$	80

38m	4-$EtOC_6H_4CH_2$	76
38n	4-MeO-3-picolyl	69
38o	4-$CNC_6H_4CH_2$	78

[a] Yield over two steps: **283** → **38** (*Scheme 49*)

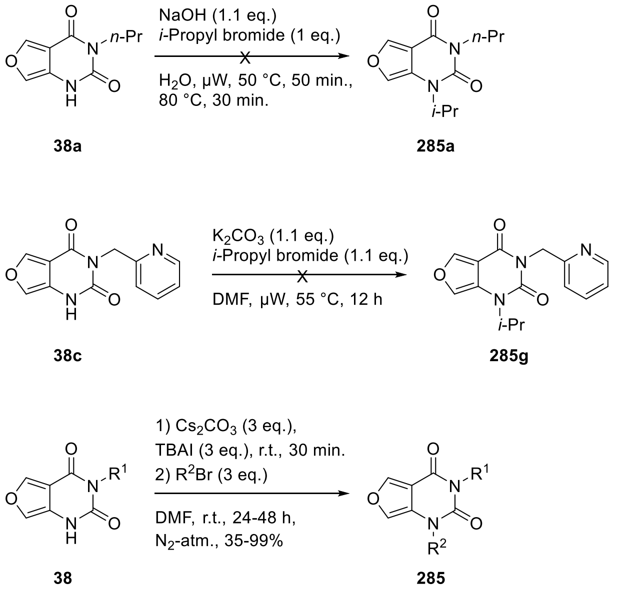
1.3 Synthesis of *N*-1/*N*-3 functionalized furo[3,4-*d*]pyrimidine-2,4-diones

Different procedures were evaluated to functionalize *N*-1. First, two different reductive *N*-alkylations were tested (Scheme 50). When **38a** was reacted with benzaldehyde, triethylsilane and trifluoroacetic acid in toluene, the formation of many side products was observed, and the desired structure **285b** was not detected.[117] Another procedure, which makes use of 1*H*-benzotriazole and benzaldehyde in a Dean-Stark set-up and sodium borohydride as a reducing agent, resulted also in many side products without the formation of **285b**.[118]



Scheme 50. Reductive alkylation procedures to functionalize N-1

Next, *N*-1 alkylation by reaction with alkyl bromides was attempted under different conditions. Reaction of **38a** with sodium hydroxide and isopropyl bromide in water under microwave conditions only resulted in formation of side products (Scheme 51).[119] When potassium carbonate was used as a base, and DMF as a solvent, the reaction with **38c** proceeded very slowly. Heating of the reaction mixture at 55 °C under microwave conditions resulted in the formation of side products and only traces of the desired compound **285g** were detected. Eventually, the use of the stronger and more soluble cesium carbonate in combination with tetrabutylammonium iodide in DMF gave good results for the alkylation reaction with isopropyl bromide.[120] Under these conditions, the furopyrimidinones **38** reacted smoothly with allyl bromide and benzyl bromide as well, and a small library of compounds **285** was constructed (Table 4). In all of these reactions, the conversion of starting material was complete and the varying yields were attributed to the occurrence of side products. The isopropylation reaction proceeded slower than the benzylation and allylation reactions, allowing more side products to be formed. This resulted in lower yields for most of the isopropylations. The use of 1.1 equivalents of sodium hydride in DMF was also evaluated to perform these alkylation reactions but this resulted in more side product formation compared to the reaction with cesium carbonate.



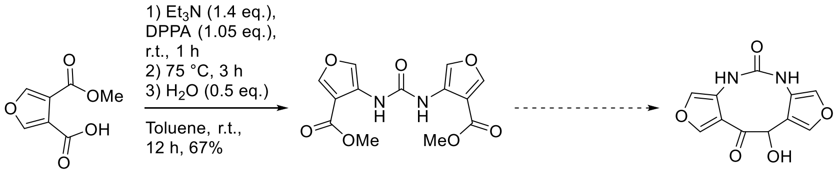
Scheme 51. Reaction of N-1 with alkyl bromides under different conditions

Table 4. Structures and yields of N-1/N-3 heterofunctionalized furo[3,4-d]pyrimidine-2,4-diones ***285***

Compound	R^1	R^2	Yield (%)
285a	*n*-Pr	*i*-Pr	55
285b	*n*-Pr	Bn	99
285c	*n*-Pr	Allyl	51
285d	Bn	*i*-Pr	66
285e	Bn	Bn	86
285f	Bn	Allyl	89
285g	2-Picolyl	*i*-Pr	35
285h	2-Picolyl	Bn	83
285i	2-Picolyl	Allyl	69
285j	4-$MeOC_6H_4CH_2$	*i*-Pr	48
285k	4-$MeOC_6H_4CH_2$	Bn	70
285l	4-$MeOC_6H_4CH_2$	Allyl	84

1.4 Further exploration of the chemistry on furo[3,4-*d*]pyrimidine-2,4-diones

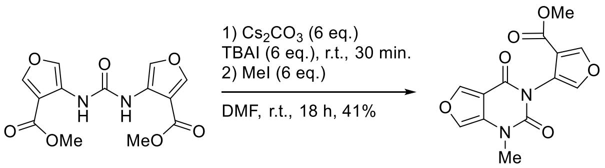
After successful functionalization of both the *N*-1 and *N*-3 nitrogens of the furo[3,4-*d*]pyrimidine-2,4-dione ring, other possibilities to explore and modify the structure were examined. While performing initial experiments on the Curtius rearrangement, small amounts of water were present and this resulted in the formation of symmetrical ureid side product **286** (Scheme 52). This compound could be a potential substrate for an acyloin condensation (after protection of the ureid nitrogen atoms) towards a 9-membered ring structure **287**. When 0.5 equivalents of water were added to the isocyanate, symmetrical ureid **286** was obtained in 67% yield.



283 **286** **287**

*Scheme 52. Synthesis of symmetrical ureid **286***

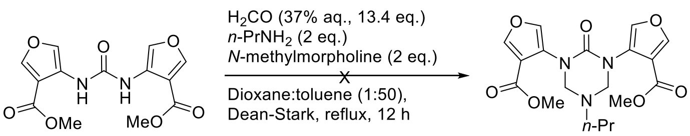
To protect the ureid nitrogens, alkylation of **286** using cesium carbonate, TBAI and iodomethane was attempted (Scheme 53). However, the major reaction product turned out to be the mono-alkylated ring closed structure **288** and was isolated in 41% yield. The double methylated ureid and the mono-methylated ureid were also present in the reaction mixture next to other unidentified side products, but isolation of these products was not successful.



286 **288**

*Scheme 53. Reaction of symmetrical ureid **286** with iodomethane*

Next, a method to protect the ureid nitrogens as a triazone moiety was evaluated. The symmetrical ureid **286** was reacted with aqueous formaldehyde and a primary amine in toluene with dioxane as co-solvent (Scheme 54).[121] Unfortunately, a complex reaction mixture was obtained and the desired triazone **289** was not detected. No further attempts were undertaken to protect the nitrogens and pursue the synthesis of a 9-membered ring structure.

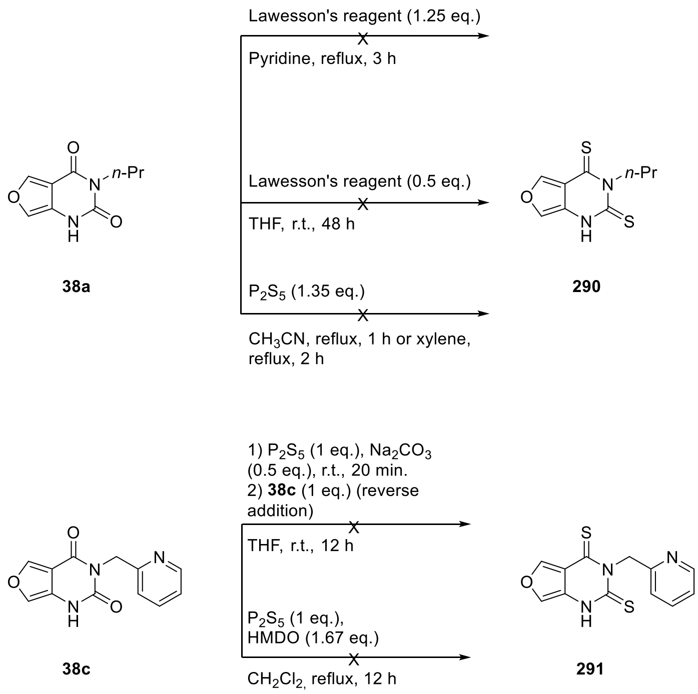


286 **289**

Scheme 54. Protection of the ureid nitrogens as a triazone moiety

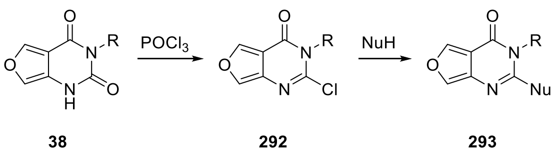
In the literature, the transformation of a pyrimidine-2,4-dione moiety to a pyrimidine-2,4-dithione has been reported.[122] First, **38a** was reacted with Lawesson's reagent under different conditions (Scheme 55). In dry pyridine under reflux conditions, a complex reaction mixture was obtained without detection of the desired compound **290**. In dry THF at room temperature, no reaction was observed. Rising the temperature and refluxing the reaction mixture for 12 hours still did not initiate any reaction. Addition of one equivalent of triethylamine and further reaction under reflux conditions only resulted in the formation of side products. Next, the use of phosphorus pentasulfide was evaluated. Reaction of **38a** with 1.35 equivalents of phosphorus pentasulfide in acetonitrile or xylene under reflux conditions only resulted in a complex reaction mixture without detection of the target compound **290**.[123,124] Phosphorus pentasulfide can be pre-activated by sodium carbonate to form an active thionating salt.[125] When this salt was reacted with **38c**, the desired compound **291** was detected in the reaction mixture, but only a small fraction of the starting product was converted after 12 hours. Prolonged stirring did not improve the conversion. Even when the experiment was repeated with 10 equivalents of phosphorus pentasulfide and 5 equivalents of sodium carbonate, a significant amount of starting product was still present after 48 hours. Finally, Curphey's thionation conditions, employing a combination of phosphorus pentasulfide and hexamethyldisiloxane, were tested.[126] These conditions gave similar results as the use of phosphorus pentasulfide and sodium carbonate. Even when 10 equivalents of phosphorus pentasulfide were used, mainly starting material was present in the reaction mixture after 12 hours.

Although two reaction conditions were found in which the desired dithione compound was formed, no full or sufficiently clean conversion was obtained and all attempts to isolate these compounds failed.



Scheme 55. Attempts to thionate the pyrimidine-2,4-dione moiety

Although the attempts to chlorinate the furo[3,4-*d*]pyrimidine-2,4-dione scaffold by treatment with phosphorus oxychloride were unsuccessful (*vide supra*), chlorination of the C-2 carbonyl group of the *N*-3 functionalized scaffold by phosphorus oxychloride was still evaluated (Scheme 56). The different reaction conditions are given in Table 5. In all cases, a complex reaction mixture was obtained and the desired compound **292** or **293** was not detected.

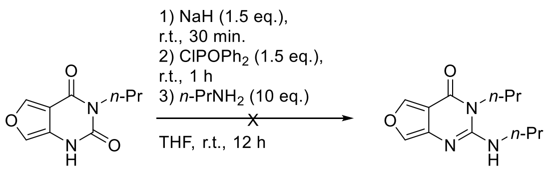


Scheme 56. Chlorination of C-2 by phosphorus oxychloride

Table 5. Reaction conditions of N-3 functionalized furo[3,4-d]pyrimidine-2,4-diones with phosphorus oxychloride

R	$POCl_3$ (eq.)	Base (equiv.)	NuH (equiv.)	T (°C)	Time (h)	Solvent
n-Pr	Large excess	/	/	reflux	3	Neat
n-Pr	3	*N*,*N*-dimethylaniline (2)	/	reflux	2	Toluene
n-Pr	1.1	DIPEA (2)	/	reflux	2.5	Toluene
n-Pr	1.1	Et_3N (2)	MeOH (10)	reflux	2	Toluene
2-picolyl	1.1	DIPEA (2)	*n*-$PrNH_2$ (10)	reflux	1	Toluene

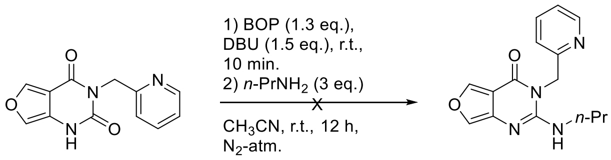
The use of other reagents, which can accomplish the same type of transformation under milder conditions, were tested. The first reagent is diphenylphosphinic chloride, which works in a very similar way as $POCl_3$ but under milder reaction conditions.[127] Compound **38a** was treated with sodium hydride in THF for 30 minutes, followed by addition of diphenylphosphinic chloride (Scheme 57). Then, an excess of *n*-propylamine was added. Unfortunately, the use of this reagent also resulted in a complex reaction mixture without detection of the C-2 chlorinated product or the desired compound **294**.



38a **294**

Scheme 57. Chlorination of N-3 functionalized furo[3,4-d]pyrimidine-2,4-dione using diphenylphosphinic chloride

Another reagent which can be used for this type of transformation under mild reaction conditions is (benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate, better known as Castro's reagent or BOP.[128] First, furopyrimidinone **38c** was activated with DBU and BOP at room temperature for 10 minutes (Scheme 58). Then, *n*-propylamine was added. LC-MS analysis of the crude reaction mixture after twelve hours showed full conversion to a complex reaction mixture, containing compound **295** and a significant amount of side products. Unfortunately, compound **295** could not be isolated.



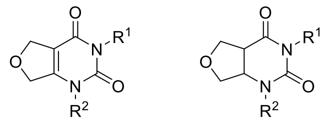
38c **295**

Scheme 58. Functionalization of C-2 by BOP-reagent

1.5 Synthesis of 5,7-dihydro- and tetrahydrofuro[3,4-*d*]pyrimidine-2,4-diones

After exploring various methods to modify the uracil/pyrimidine moiety of the scaffold (*vide supra*), research efforts turned towards altering the furan ring. A literature search revealed that the compound classes of *N*-1/*N*-3 functionalized 5,7-dihydropyrimidine-2,4-diones **296** and tetrahydrofuropyrimidine-2,4-diones **297** (Figure 12) were highly underexplored. With a straightforward pathway to the furo[3,4-

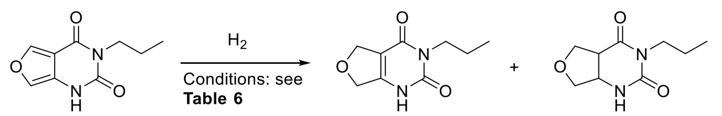
d]pyrimidine-2,4-diones in hand, a selective hydrogenation of the furan ring to get the respective di- or tetrahydrofuro[3,4-*d*]pyrimidine-2,4-diones looked like an interesting approach to investigate.



296 **297**

Figure 12. The unexplored compound classes of 5,7-dihydropyrimidine-2,4-diones and tetrahydrofuropyrimidine-2,4-diones

The first hydrogenation reactions were performed on furopyrimidinone **38a** (Scheme 59). Rhodium on alumina is generally known for its high activity for the hydrogenation of aromatic rings, while palladium on carbon has only a low activity for hydrogenation of aromatics.[129] Therefore, both of these catalysts were evaluated. When 5 mol% of palladium (10 wt% on activated carbon) was used under an atmosphere of 1 bar of hydrogen pressure, full conversion of starting material was observed after four hours (Table 6, entry 1). The major reaction product was 5,7-dihydrofuro[3,4-*d*]pyrimidine-2,4-dione **298** and was isolated in 66% yield. The minor reaction product was tetrahydrofuro[3,4-*d*]pyrimidine-2,4-dione **299** (not isolated). When the hydrogen pressure was increased to 5 bar, full conversion of starting material was observed after one hour, and the dihydrofuropyrimidinone **298** was isolated in 71% yield (Table 6, entry 2). When 5 mol% of rhodium (5 wt% on alumina) was used as a catalyst, full conversion of starting material and of the dihydrofuropyrimidinone **298** was achieved only after twelve hours under a hydrogen atmosphere of 5 bar (Table 6, entry 3). The tetrahydrofuropyrimidinone **299** was isolated in 79% yield.



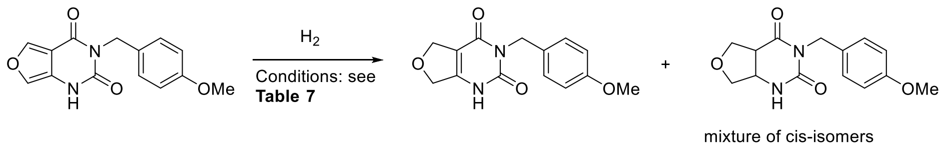
38a **298** **299**

*Scheme 59. Hydrogenation of furopyrimidinone **38a***

*Table 6. Reaction conditions and yields of the hydrogenation reaction of **38a**. 5 mol% of catalyst was used. H_2 pressure is expressed as overpressure relative to atmospheric pressure in bar. All reactions were carried out at room temperature in methanol as a solvent.*

Entry	Catalyst	H_2 (bar)	t (h)	Yield of **298** or **299** (%)
1	10 wt% Pd/C	1	4	66 (**298**)
2	10 wt% Pd/C	5	1	71 (**298**)
3	5 wt% Rh/Al	5	12	79 (**299**)

Since the use of palladium on carbon resulted in full hydrogenation of the furan ring to some extent, a screening of different in-house hydrogenation catalysts was performed with the aim to find a catalyst which showed complete selectivity for the dihydrofuropyrimidinone product. Compound **38d**, with a *para*-methoxybenzyl (PMB) moiety on *N*-3, was selected as the starting material for this screening (Scheme 60). This way, any undesired hydrogenolytic activity of the catalyst would be observed by the occurrence of *N*-3 deprotected furo[3,4-*d*]pyrimidine-2,4-dione. The results are summarized in Table 7. Except for Adam's catalyst (entry 2), all of the catalysts afforded more dihydrofuropyrimidinone **300** than tetrahydrofuropyrimidinone **301** (entries 1, 3-6). The use of Adam's catalyst also resulted in the formation of many unidentified side products, while no differences in the formation of side products could be detected for the other catalysts. Lindlar's catalyst gave the best dihydro/tetrahydro ratio, but conversion of the starting material was very slow (entry 4). Pearlman's catalyst (entry 5) and the regular palladium on activated carbon (entry 6) combined a good conversion of starting material with a relatively good dihydro/tetrahydro ratio. From this screening, it was concluded that 10 wt% of palladium on activated carbon was the most convenient catalyst for the hydrogenation of furo[3,4-*d*]pyrimidine-2,4-dione **38d** to 5,7-dihydro[3,4-*d*]pyrimidine-2,4-dione **300**. No hydrogenolysis of the PMB group was observed in any of the reactions.



38d **300** **301**

*Scheme 60. Catalyst screening for the hydrogenation of furopyrimidinone **38d***

*Table 7. Catalyst screening for the hydrogenation reaction of **38d**. 5 mol% of catalyst was used. All reactions were equipped with a balloon filled with pure hydrogen gas. All reactions were carried out at room temperature in methanol as a solvent.*

Entry	Catalyst	t (h)	**38d**:**300**:**301**[a]	Ratio **300**/**301**[a]
1	3.5 wt% Pb/5 wt% Pd/$CaCO_3$	1	57:35:8	4.4
2	PtO_2 (Adam's catalyst)	1	56:17:27	0.6
3	5 wt% Pd/$BaSO_4$	1	58:37:5	7.4
4	5 wt% Pd/$CaCO_3$, poisoned (Lindlar's catalyst)	1	91:8:1	8
5	20 wt% $Pd(OH)_2$/C	1	28:59:13	4.5
6	10 wt% Pd/C	1	25:64:11	5.8

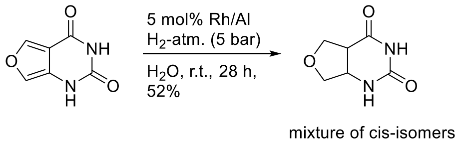
[a]: Estimated by LC-MS analysis

Since the screening did not result in a better catalyst for the synthesis of 5,7-dihydrofuropyrimidinones, compounds **300** and **301** were produced under the same reaction conditions as the *n*-propyl derivative **38a** and were obtained in 66% and 71% yield, respectively (Table 8).

*Table 8. Reaction conditions and yields of the hydrogenation reaction of **38d**. 5 mol% of catalyst was used. H_2 pressure is expressed as overpressure relative to atmospheric pressure in bar. All reactions were carried out at room temperature in methanol as a solvent.*

Entry	Catalyst	H_2 (bar)	t (h)	Yield of **300** or **301** (%)
1	10 wt% Pd/C	5	1	66 (**300**)
2	5 wt% Rh/Al	5	12	71 (**301**)

The tetrahydrofuro[3,4-*d*]pyrimidine-2,4-dione scaffold **323** was synthesized by heterogeneous catalytic hydrogenation of the furo[3,4-*d*]pyrimidine-2,4-dione scaffold **6** (Scheme 61). Five mole percent of rhodium in the form of 5 wt% rhodium on alumina was used as the heterogeneous catalyst, and water was used as the solvent. After stirring for twenty-eight hours under a hydrogen atmosphere of 5 bar, complete conversion of the starting material was observed and the scaffold **323** was isolated in 52% yield.



6 **323**

Scheme 61. Tetrahydrofuro[3,4-d]pyrimidine-2,4-dione was obtained via Rhodium-catalyzed hydrogenation of furo[3,4-d]pyrimidine-2,4-dione

X-ray analysis was performed on the crystals of **323** in order to determine the stereochemistry (Figure 13). The crystals were composed of the two cis-isomers in a 1:1 ratio. One cis-isomer has C1 (S) and C4 (R) stereochemistry, the other one has C1 (R) and C4 (S).

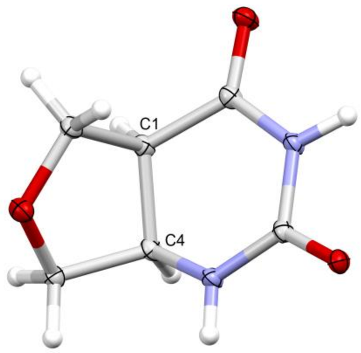
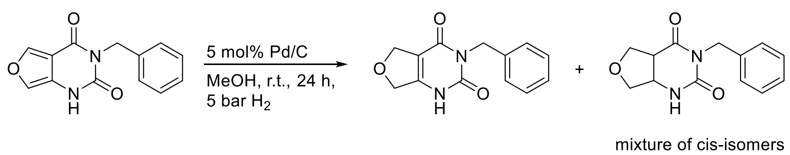


*Figure 13. Structure of the C1 (S) C4 (R) stereoisomer of tetrahydrofuro[3,4-d]pyrimidine-2,4-dione **323**. Thermal displacement ellipsoids are shown at the 50% probability level.*

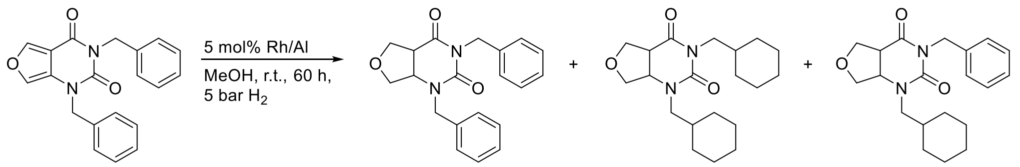
The hydrogenation procedure with a palladium on carbon catalyst proved to be compatible with a PMB group on *N*-3. However, because this palladium catalyst is known for its high hydrogenolytic activity,[129] the stability of a benzyl substituent on *N*-3 under palladium catalyzed hydrogenation reaction conditions was also evaluated. When compound **38b** was subjected to 5 bar of hydrogen pressure for twenty-four hours using 5 mol% of palladium on carbon, no debenzylation was observed (Scheme 62). Based on LC-MS, both **302** and **303** were present in the reaction mixture in a 1:1 ratio, from which product **302** was isolated in 36% yield.



38b **302**, 36% **303**, not isolated

Scheme 62. Palladium catalyzed hydrogenation of a N-3 benzylated furopyrimidinone

When the *N*-1/*N*-3 dibenzylated furopyrimidinone **285e** was hydrogenated with rhodium on alumina, full conversion of the starting material was observed after twelve hours (Scheme 63). However, LC-MS analysis indicated that hydrogenation of the benzyl rings had occurred and that the expected product **304a** was present in the reaction mixture together with more saturated products. The predominant species were **305** (50%), **304a** (25%) and **304b** (8%), while other species -with saturation degrees ranging from **304a** to **304b**- were present in a total amount of 17%. After 60 hours of total reaction time, the amount of product **304a** decreased to 12%, while **304b** and **305** increased to 18% and 55%, respectively. The reaction was terminated and only compound **305** could be isolated from the reaction mixture in 20% yield. The structure of compound **305** was elucidated by using a combination of 1D-NMR and 2D-NMR techniques. The apparent selectivity for the hydrogenation of the *N*-1 benzyl moiety may be due to electronic and/or steric effects. No debenzylation products were observed during the reaction.

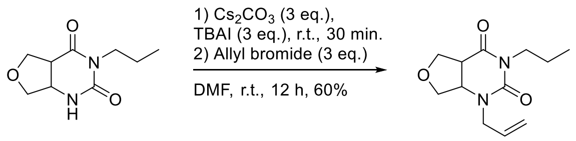


mixture of cis-isomers | mixture of cis-isomers | mixture of cis-isomers

285e | **304a,** not isolated | **304b,** not isolated | **305**, 20%

*Scheme 63. Rhodium catalyzed hydrogenation of **285e** affected the benzyl group on N-1*

One of the tetrahydrofuropyrimidinone compounds was alkylated following the same procedure as for the furopyrimidinones **38**. When **299** was reacted with cesium carbonate, tetrabutylammonium iodide and allyl bromide, the desired product **306** was obtained in 60% yield (Scheme 64).



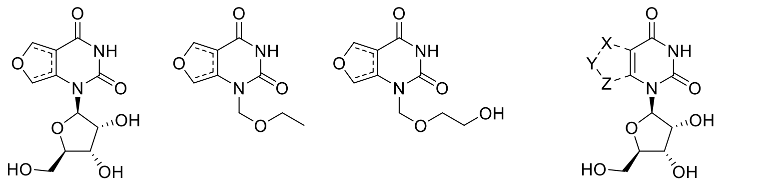
mixture of cis-isomers

299 | **306**

*Scheme 64. Alkylation of tetrahydrofuropyrimidinone **299** with allyl bromide*

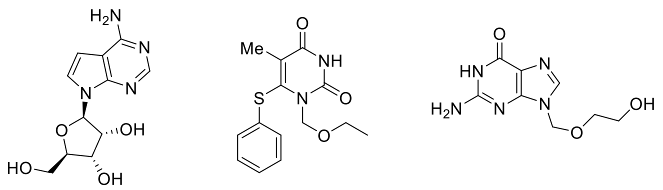
1.6 Synthesis of *N*-1 functionalized furo[3,4-*d*]pyrimidine-2,4-diones

Three different substituents were proposed for the *N*-1 functionalization of the furo[3,4-*d*]pyrimidine scaffolds in order to obtain nucleoside analogues **307-309** (Figure 14). The antiviral drugs Idoxuridine **11**, Trifluridine **12** and Zidovudine **13** all possess a 2-deoxyribose moiety (Figure 4), while the antibacterial agent Tubercidin **311a** is equipped with a ribose moiety (Figure 14). The (stereoselective) synthesis of 2-deoxyglycosides is often more complex compared to the corresponding glycosides.[130] The synthesis of the proposed glycoside **307** requires only a straightforward glycosylation reaction with inexpensive 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (ATBR), followed by a deprotection reaction. Moreover, the field of 2-deoxyribose-based nucleoside analogues has been investigated extensively, while a literature search on the 5-membered ring annulated uridine substructure **310** indicated that only seventeen such structures have been reported so far, and only four of them were involved in biological studies.[131–134] Therefore, the biological evaluation of this type of uridine analogues would be very interesting. The acyclic nucleoside analogues **308** and **309** were proposed based on literature reports on the antiviral and antifungal activity of acyclouridine derivatives.[135–137] For example, compound **311b** inhibited the replication of the HIV-1 virus in MT-4 cells *in vitro*, exhibiting an EC_{50} value of 0.33 µM.[137] In addition, Aciclovir (**311c**, Figure 14) is a well-known example of an acyclic nucleoside analogue drug.



X, Y, Z = any atom except H

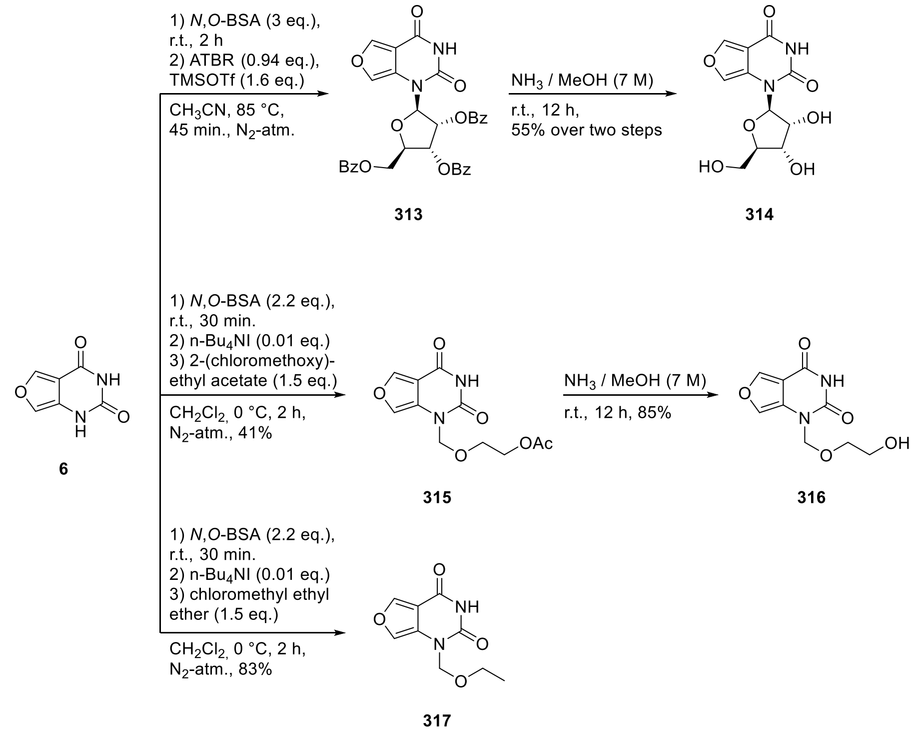
307 **308** **309** **310**



311a, Tubercidin **311b** **311c**, Aciclovir

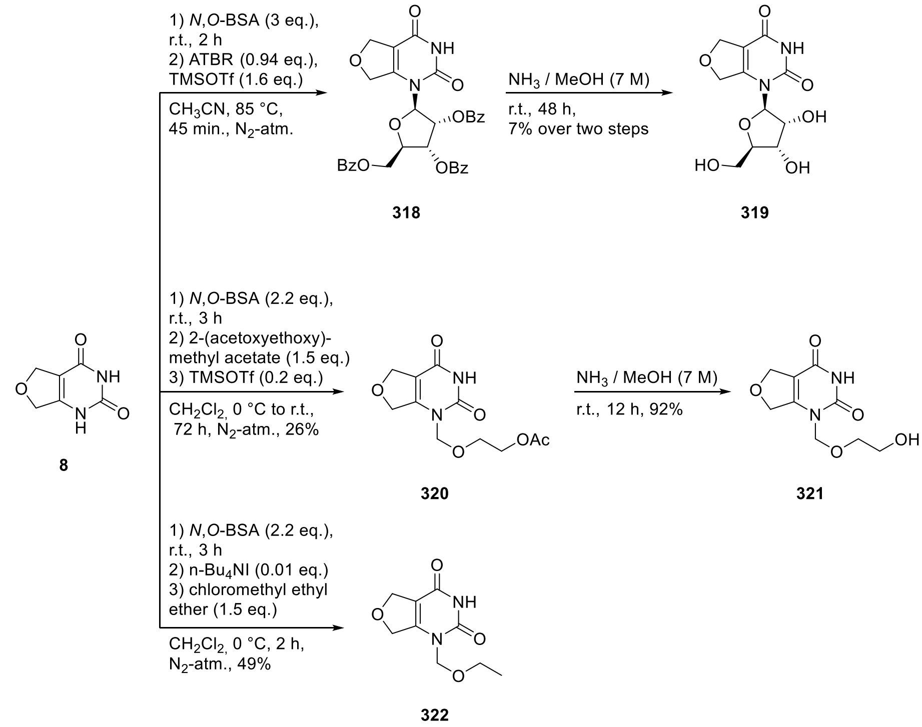
*Figure 14. Proposed structures of nucleoside analogues **307-309**, the SciFinder search substructure **310**, the structure of antibacterial Tubercidin **311a** and the antiviral compounds **311b-c***

Three different *N*-1 functionalized furo[3,4-*d*]pyrimidine-2,4-diones were synthesized following literature procedures that describe the derivatization of uracil (Scheme 65). The furo[3,4-*d*]pyrimidine-2,4-dione scaffold **6** was glycosylated under Vörbruggen conditions.[138] The scaffold was first silylated by reaction with three equivalents of *N*,*O*-bis(trimethylsilyl)acetamide (*N*,*O*-BSA) in acetonitrile at room temperature for two hours, followed by addition of 0.94 equivalents of ATBR and 1.6 equivalents of trimethylsilyl trifluoromethanesulfonate and heating at 85 °C for 45 minutes. The benzoyl-protected structure **313** was subsequently deprotected by treatment with methanolic ammonia at room temperature for twelve hours, to afford the glycosylated furo[3,4-*d*]pyrimidine-2,4-dione **314** in 55% yield over two steps. The functionalization of *N*-1 with 2-(chloromethoxy)ethyl acetate was also initiated by silylation of the scaffold, but under slightly different reaction conditions.[139] The silylation was followed by addition of a catalytic amount of tetrabutylammonium iodide at room temperature and 1.5 equivalents of 2-(chloromethoxy)ethyl acetate at 0 °C. After stirring at 0 °C for two hours, the reaction was finished and the acylated structure **315** was isolated in 41% yield. Deprotection of the hydroxyl group was achieved by methanolic ammonia to give compound **316** in 85% yield. In a similar fashion, scaffold **6** reacted with chloromethyl ethyl ether to afford structure **317** in 83% yield.



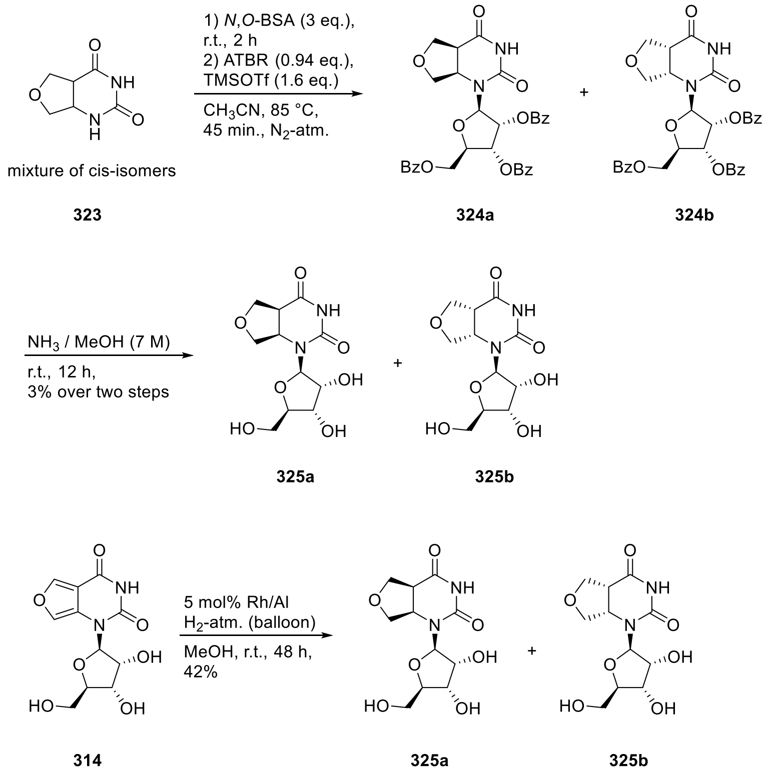
Scheme 65. Synthesis of N-1 functionalized furo[3,4-d]pyrimidine-2,4-diones

The 5,7-dihydrofuro[3,4-*d*]pyrimidine-2,4-dione scaffold **8** (Scheme 66) was synthesized following a literature procedure.[34] This scaffold was then glycosylated under the Vörbruggen conditions which have been described earlier. However, a significant amount of side products was formed, and the desired glycosylated structure **319** was isolated in only 7% yield over two steps. For the synthesis of the 5,7-dihydro analogue of **315**, another procedure was followed in an attempt to improve the yield of the first reaction step.[140] Thus, after silylation of **8** with *N*,*O*-bis(trimethylsilyl)acetamide, 1.5 equivalents of 2-(acetoxyethoxy)methyl acetate were added at room temperature, followed by 0.2 equivalents of trimethylsilyl trifluoromethanesulfonate at 0 °C. After stirring at room temperature for three days, the reaction was finished and the acylated compound **320** was isolated in 26% yield. Deprotection of the hydroxyl group by methanolic ammonia gave derivative **321** in 92% yield. The reaction of **8** with chloromethyl ethyl ether was also characterized by a lower yield compared to its analogue **6**, and compound **322** was obtained in 49% yield.



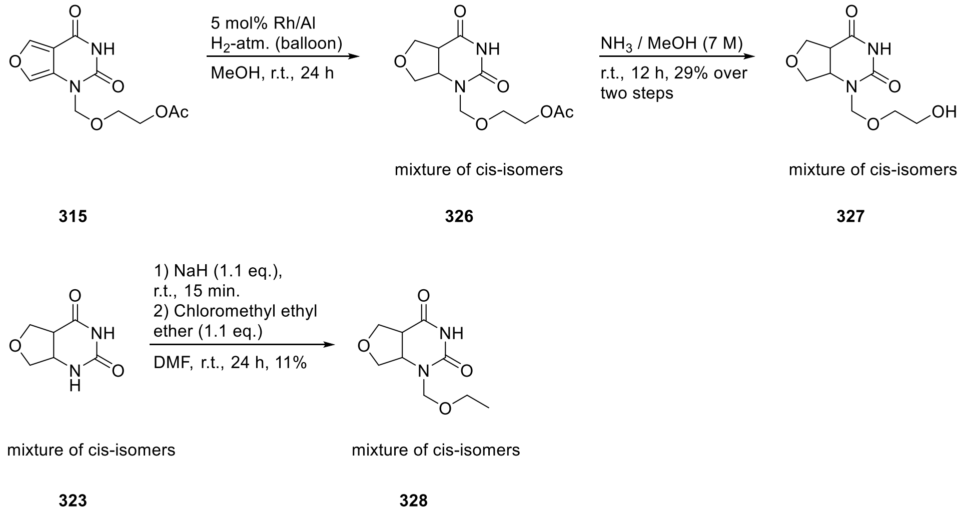
Scheme 66. Synthesis of N-1 functionalized 5,7-dihydrofuro[3,4-d]pyrimidine-2,4-diones

The synthesis of the *N*-1 glycosylated tetrahydrofuro[3,4-*d*]pyrimidine-2,4-dione **325** under Vörbruggen reaction conditions was accompanied by the formation of many side products, and the desired compound **325** was obtained in only 3% yield over two steps as two diastereomers **325a** and **325b** in a 1:1 ratio (Scheme 67). Hydrogenation of the *N*-1 glycosylated furo[3,4-*d*]pyrimidine-2,4-dione **314** using five mole percent of rhodium on alumina in methanol for two days afforded product **325** in 42% yield as two diastereomers **325a** and **325b** in a 1:1 ratio. Due to the low-pressure (up to 5 bar) hydrogenator being unavailable for a long time, a balloon filled with hydrogen gas was used instead. The two isolated diastereomers **325a** and **325b** were obtained as colourless viscous oils, so their absolute stereochemistry could not be determined *via* X-ray diffraction spectroscopy.



*Scheme 67. The synthesis of N-1 glycosylated tetrahydrofuro[3,4-d]pyrimidine-2,4-dione **325** via two different pathways*

The synthesis of the *N*-1 functionalized tetrahydrofuro[3,4-*d*]pyrimidine-2,4-dione **327** was also achieved *via* hydrogenation of the furan ring (Scheme 68). This time, the acylated furo[3,4-*d*]pyrimidine analogue **315** was chosen as a precursor for **327** instead of the deprotected product. This approach allowed for a purification step by silica gel chromatography on the relatively apolar compound **326**. Most of the impurities, which are formed in the first reaction step, could be removed on silica gel and the use of preparative HPLC, which consumes high volumes of HPLC-grade solvents, could be avoided to isolate the polar product **327**. Compound **327** was isolated in 29% yield over two steps. The synthesis of this compound was also attempted under the reaction conditions as described by Sun and coworkers,[139] but many side products were formed and the product could not be isolated from the reaction mixture. The same problem was observed when the synthesis of compound **328** was attempted under the reaction conditions as reported by Sun *et al*. However, treatment of tetrahydrofuro[3,4-*d*]pyrimidine-2,4-dione **323** with sodium hydride in DMF at room temperature for fifteen minutes and subsequent reaction with chloromethyl ethyl ether resulted in a smaller but still significant amount of side products, and product **328** was obtained in 11% yield.



*Scheme 68. The synthesis of N-1 functionalized tetrahydrofuro[3,4-d]pyrimidine-2,4-diones **327** and **328***

Compound **327** was obtained in the form of white crystals, and its absolute configuration was determined *via* X-ray diffraction spectrometry (Figure 15). The crystals contained the two cis-isomers in a 1:1 ratio. One cis-isomer has C1 (R) and C4 (S) stereochemistry, the other one has C1 (S) and C4 (R).

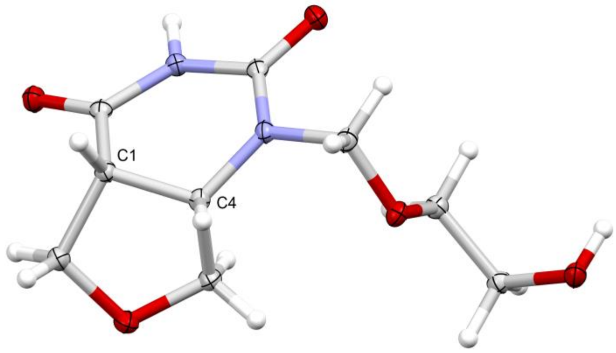


*Figure 15. Structure of the C1 (R) C4 (S) stereoisomer of N-1 functionalized tetrahydrofuro[3,4-d]pyrimidine-2,4-dione **327**. Thermal displacement ellipsoids are shown at the 50% probability level.*

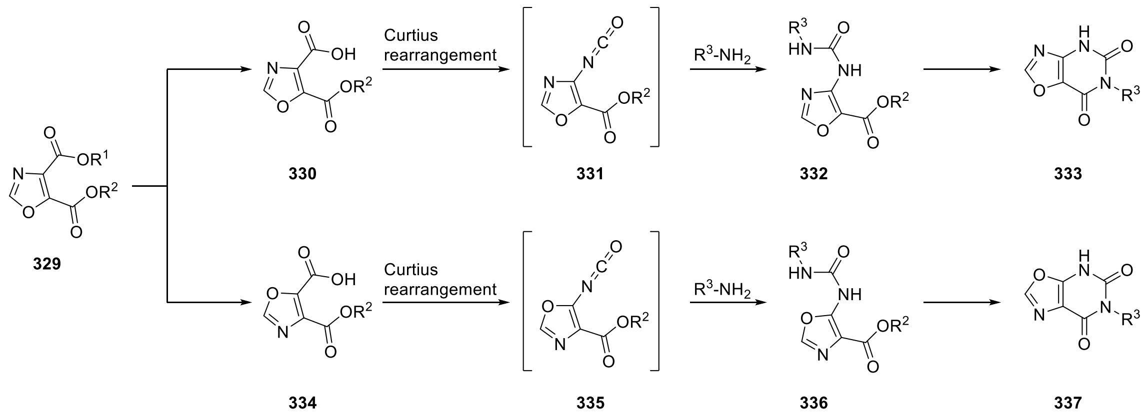
1.7 Conclusions

In summary, a straightforward synthetic route was developed to synthesize the furo[3,4-*d*]pyrimidine-2,4-dione scaffold, using a Curtius rearrangement as the key step. Attempts to convert these furopyrimidinones into the desired furo[3,4-*d*]pyrimidines were not successful, due to the reactivity of the furan ring towards undesired side product formation upon treatment with phosphorus oxychloride. Instead, the furo[3,4-*d*]pyrimidine-2,4-diones were further explored and developed by functionalization of the *N*-3 and *N*-1 nitrogen atoms. Hydrogenation of the furan ring of the furo[3,4-*d*]pyrimidine-2,4-diones gave access to the 5,7-dihydrofuro[3,4-*d*]pyrimidine-2,4-diones and the tetrahydrofuro[3,4-*d*]pyrimidine-2,4-diones. This resulted in the construction of a library of 45 compounds.

Parts of this chapter have been published in the journal Synthesis.[141]

2 Synthesis of oxazolo[4,5-*d*]pyrimidines and oxazolo[5,4-*d*]pyrimidines

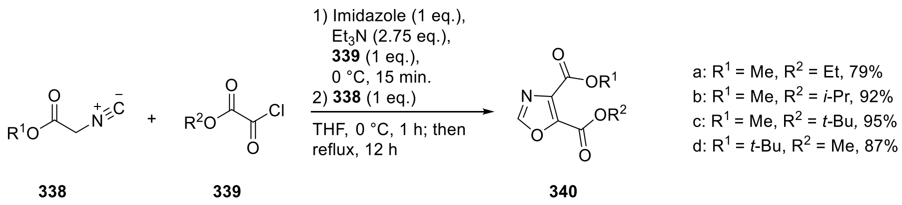
The proposed pathway for the synthesis of oxazolo[4,5-*d*]pyrimidine and oxazolo[5,4-*d*]pyrimidine analogues was based on the successfully developed pathway towards furo[3,4-*d*]pyrimidine analogues. Synthesis of the furo[3,4-*d*]pyrimidine pathway started from commercially available dialkyl furan-3,4-dicarboxylate and was followed by the hydrolysis of one of the two ester groups to give the monocarboxylic acid. This hydrolysis step did not need to be selective for the C-3 methyl ester over the C-4 methyl ester due to the symmetry of the starting material. However, dialkyl oxazole-4,5-dicarboxylates are asymmetric and conversion of one of the ester groups to a monocarboxylic acid should be selective for either position 4 or 5 to avoid the formation of isomers. Thus, starting from dialkyl ester **329**, a chemoselective transformation step should give the mono-acid **330** or **334** (Scheme 69). A Curtius rearrangement on the carboxylic acid would then give the corresponding isocyanate, which would afford ureid compounds **332** or **336** upon reaction with an appropriate amine. Ring closure of the ureid structures would then result in the oxazolo[4,5-*d*]pyrimidine-5,7-diones **333** or oxazolo[5,4-*d*]pyrimidine-5,7-diones **337**, respectively. Chlorination of these scaffolds by phosphorus oxychloride and further modification could then give synthetic access to the C2/C4-substituted oxazolo[4,5-*d*]pyrimidines and oxazolo[5,4-*d*]pyrimidines.[56]



Scheme 69. Proposed synthetic pathways towards oxazolo[4,5-d]pyrimidine analogues (top) and oxazolo[5,4-d]pyrimidine analogues (bottom)

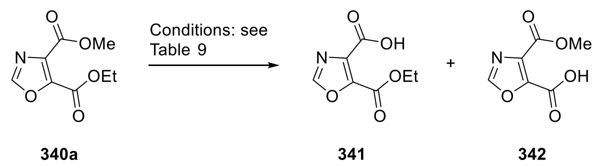
2.1 Synthesis of the monocarboxylic acid precursors for the Curtius rearrangement reaction

In order to accomplish the chemoselective conversion of either the C-4 or C-5 ester group to a carboxylic acid, R^1 should be different from R^2 in structure **329**. In the literature, the synthesis of dimethyl- and diethyl oxazole-4,5-dicarboxylate was already described, and one of the reported methods seemed likely to be able to deliver the desired esters (with $R^1 \neq R^2$) after appropriate adjustments.[142–145] Indeed, following the reported protocol of Henneke *et al.*, reaction of commercially available ethyl 2-chloro-2-oxoacetate **339a**, imidazole, triethylamine and commercially available methyl isocyanoacetate **338a** afforded 5-ethyl 4-methyl oxazole-4,5-dicarboxylate **340a** in 79% yield (Scheme 70).[145] Isopropyl 2-chloro-2-oxoacetate **339b** and *tert*-butyl 2-chloro-2-oxoacetate **339c** were easily prepared by reacting oxalyl chloride with isopropanol and *tert*-butanol, respectively.[146,147] Reaction of these alkyl 2-chloro-2-oxoacetate products with methyl isocyanoacetate gave 5-isopropyl 4-methyl oxazole-4,5-dicarboxylate **340b** and 4-methyl 5-*tert*-butyl oxazole-4,5-dicarboxylate **340c** in very good yields. Reaction of both commercially available methyl 2-chloro-2-oxoacetate **339d** and *tert*-butyl isocyanoacetate **338d** afforded 5-methyl 4-*tert*-butyl oxazole-4,5-dicarboxylate **340d** in 87% yield.



Scheme 70. Synthesis of dialkyl oxazole-4,5-dicarboxylates

First, the chemoselective conversion of compound **340a**, bearing a methyl and ethyl ester group, was investigated. The use of this compound would be cheaper and more atom-economical than the use of the respective isopropyl or *tert*-butyl ester molecules **340b-c**. When compound **340a** was reacted with one equivalent of lithium hydroxide in THF-H_2O, no full selectivity was observed but the methyl ester **342** was formed predominantly (Scheme 71, Table 9, entry 1). Attempts to separate compound **342** from **341** were not successful. A selective system, using cyanide in hexamethylphosphoric triamide (HMPT) as a solvent, was reported by Müller and Siegfried.[148] Under these reaction conditions, no full selectivity was observed either, but this time the ethyl ester turned out to be the major reaction product (entry 2). In a last experiment, HMPT was replaced by DMF for two reasons. First of all, HMPT is suspected of being carcinogenic, and secondly it is more difficult to remove during work-up than DMF.[149] In DMF, the reaction proceeded more slowly and less selectively compared to HMPT. Unfortunately, monocarboxylic acid **341** could not be separated from **342** in any of the experiments. In order to obtain either compound **341** or **342** in its pure form, a conversion method with complete chemoselectivity was required.



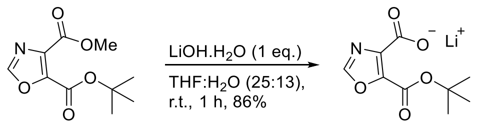
*Scheme 71. Conversion of diester **340a** into a monocarboxylic acid*

*Table 9. Reaction conditions of the conversion of diester **340a** into a monocarboxylic acid*

Entry	Reagent	Solvent	T (°C)	t (h)	Ratio **340a:341:342**[a]
1	LiOH (1 eq.)	THF:H_2O (7:4)	0 °C to r.t.	1	5:19:76
2	KCN (1.4 eq.)	HMPT	75	62	7:90:3
3	KCN (2.1 eq.)	DMF	75	64	15:80:5

[a]: Product ratios were estimated by LC-MS

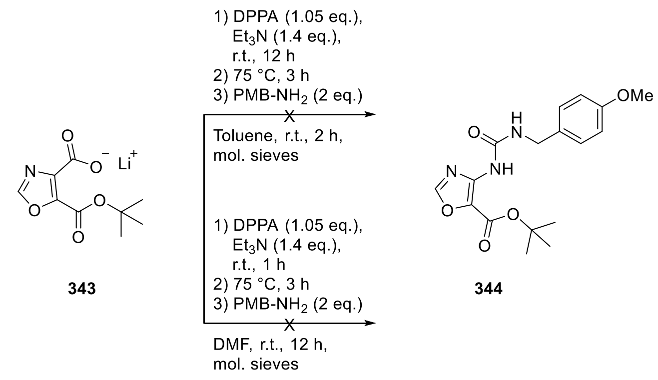
No complete discrimination between the ethyl and methyl ester group could be achieved and therefore, the next experiment was carried out on the *tert*-butyl ester **340c** (Scheme 72). The chemoselective base-catalyzed hydrolysis of a methyl ester function in the presence of a tert-butyl ester group has already been described by White *et al*.[150] When these reaction conditions were applied to the hydrolysis of **340c**, a complete and fully selective conversion towards lithium salt **343** was observed after one hour. Unfortunately, all attempts to isolate compound **343** in its free acid form were unsuccessful due to a very high instability of the *tert*-butyl ester group by acidification. Therefore, the subsequent Curtius rearrangement was attempted on the lithium carboxylate salt **343** (*vide infra*), which was isolated in 86% yield. To anticipate on solubility problems of this lithium salt, the synthesis of the cesium salt was also considered in order to improve its solubility.[151] Reaction of **340c** with one equivalent of cesium hydroxide in THF:H_2O gave a complete and full conversion to cesium 5-(*tert*-butoxycarbonyl)oxazole-4-carboxylate after one hour. However, the extreme hygroscopic nature of the obtained cesium salt made it very difficult to isolate the salt as a water-free compound, and the presence of water during the Curtius rearrangement would be problematic.



340c **343**

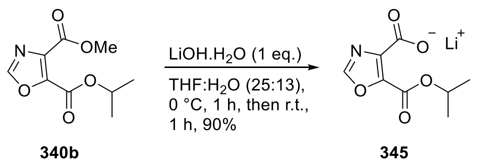
*Scheme 72. Chemoselective hydrolysis of diester **340c** using lithium hydroxide*

The lithium 5-(*tert*-butoxycarbonyl)oxazole-4-carboxylate **343** was used to perform a Curtius rearrangement towards ureid compound **344** (Scheme 73). The reaction conditions for the Curtius rearrangement, which were previously used for the synthesis of furo[3,4-*d*]pyrimidine-2,4-diones, were slightly adjusted. Due to the limited solubility of the lithium salt **343** in the reaction mixture, the reaction time of the first step was extended to twelve hours. Molecular sieves were added to the reaction mixture to capture any water originating from the hygroscopic lithium salt. Then, the mixture was heated at 75 °C for three hours. A successful Curtius rearrangement is characterized by the evolution of gas bubbles in this step, but no clear formation of gas bubbles was observed during this stage. Two equivalents of 4-methoxybenzylamine were added, and after stirring for two hours at room temperature, LC-MS analysis showed that no conversion of starting material had occurred. One possible explanation for this observation could be the very poor solubility of the lithium salt in the reaction mixture. Therefore, toluene was replaced by DMF as a solvent in a next attempt. In DMF, the solubility of the lithium salt was much better, and the reaction time of the first step was reduced again to its usual value of one hour (Scheme 73). During the heating step, no evolution of gas bubbles was observed again. After addition of the amine and stirring at room temperature, LC-MS analysis showed that a complex reaction mixture had formed in which no starting material was present anymore. Based on these results, the lithium salt was considered as an inappropriate starting material for the Curtius rearrangement, and the focus turned again towards acquiring the monocarboxylic acid.



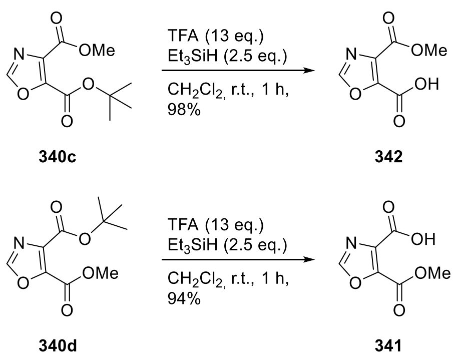
*Scheme 73. Attempts to perform a Curtius rearrangement on lithium salt **343***

A possible solution to obtain the monocarboxylic acid could be the replacement of the *tert*-butyl ester group by an isopropyl ester function, as the latter is expected to be more stable against acidification. When isopropyl ester **340b** was reacted with one equivalent of lithium hydroxide, complete chemoselectivity was observed again and lithium 5-isopropyloxazole-4-carboxylate **345** was obtained in 90% yield (Scheme 74). Unfortunately, all attempts to isolate compound **345** in its free acid form were unsuccessful, due to the instability of the isopropyl ester group during acidification.



*Scheme 74. Chemoselective hydrolysis of diester **340b** using lithium hydroxide*

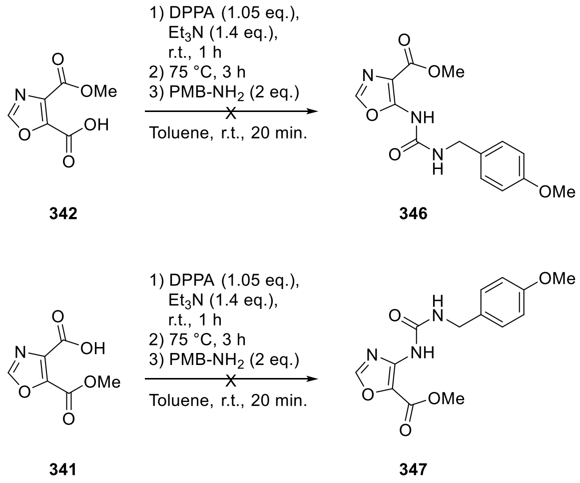
The observed instability of the tert-butyl group against acidification forms the basis for the well-known acidic deprotection of the BOC protecting group.[152] Therefore, the acid mediated deprotection of the *tert*-butyl ester group was attempted. Reaction of the *tert*-butyl ester **340c** or **340d** with an excess of trifluoroacetic acid and 2.5 equivalents of triethylsilane afforded the corresponding monocarboxylic acid **342** or **341**, respectively, in nearly quantitative yields (Scheme 75).[153]



*Scheme 75. TFA-mediated deprotection of tert-butyl esters **340c** and **340d***

2.2 Evaluation of the Curtius rearrangement reaction

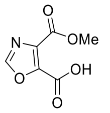
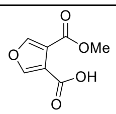
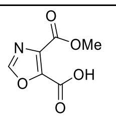
With the free monocarboxylic acids **342** and **341** in hand, the Curtius rearrangement was attempted again. Following the usual protocol, **342** and **341** were first reacted with diphenyl phosphoryl azide and triethylamine at room temperature for one hour and then heated at 75 °C for three hours to affect the rearrangement (Scheme 76). No evolution of gas bubbles was observed during the heating step. Twenty minutes after addition of the amine to the reaction mixture, LC-MS analysis showed the presence of the starting materials **342** or **341**, unreacted DPPA and unidentified side products. In both reactions, formation of the desired ureids **346** or **347** could not be detected. Therefore, the course of this reaction was studied in more detail.



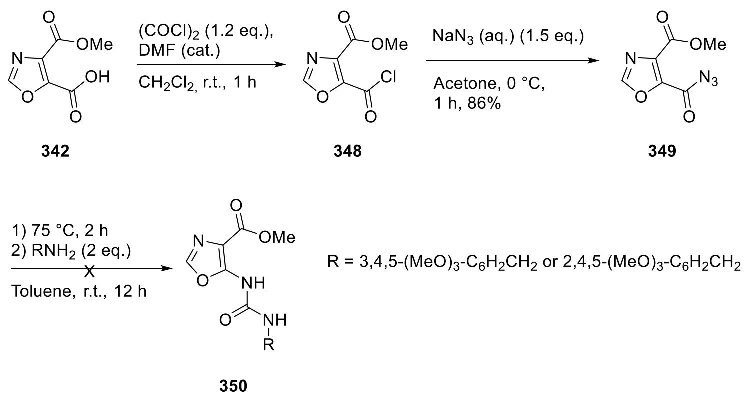
*Scheme 76. The Curtius on monocarboxylic acids **342** and **341** was unsuccessful*

In dichloromethane instead of toluene, 4-(methoxycarbonyl)oxazole-5-carboxylic acid did not react with diphenyl phosphoryl azide after one hour at room temperature and after one hour at 50 °C (Table 10, entry 1). Heating of the reaction mixture at 75 °C for twelve hours resulted in formation of a complex reaction mixture. The carboxylic acid was consumed, and only a trace of DPPA was still present. After addition of 4-methoxybenzylamine, the desired ureid could not be detected in the reaction mixture. Replacing the combination of triethylamine and dichloromethane by pyridine gave a very similar course of the reaction (entry 2). At room temperature and 50 °C, no reaction was observed. Heating at 75 °C for twelve hours resulted in consumption of the carboxylic acid and the majority of DPPA, and formation of a complex reaction mixture. After addition of the amine, formation of the desired ureid was not observed. This was in contrast to the reference reaction of 4-(methoxycarbonyl)furan-3-carboxylic acid in toluene, which reacted smoothly with DPPA at room temperature (entry 3). After heating at 75 °C for twelve hours and addition of the amine, a successful formation of the desired ureid was observed. The reaction of the furan starting material with DPPA also proceeded very well in acetonitrile as a solvent at room temperature (entry 4), while 4-(methoxycarbonyl)oxazole-5-carboxylic acid failed to react under these conditions (entry 5). Thus, no reaction occurred at room temperature or at 50 °C, while heating of the reaction mixture at 75 °C lead to undesired reactions (entries 1, 2 and 5). The onset temperature of the thermal degradation of neat DPPA is reported to be around 270 °C.[154] Therefore, thermal degradation of DPPA is not expected to cause the undesired reactions at 75 °C. However, the thermal ring-opening of certain oxazoles has been reported in the literature at 95 °C.[155] This could be a possible explanation for the occurrence of side reactions at elevated temperature. Besides thermal ring-opening, base-induced ring-opening of the oxazole could also take place. Vedejs and Monahan reported a method to prevent base-induced ring opening of oxazoles by treatment with a Lewis acid.[156] Thus, one equivalent of borane-THF complex was added to the reaction at room temperature, and the temperature was then raised to 75 °C for two hours (entry 5). Unfortunately, these reaction conditions also lead to formation of a complex mixture, and after addition of the amine, the desired ureid could not be detected.

Table 10. Study on the reaction of monocarboxylic acid starting materials with DPPA and the subsequent Curtius rearrangement. The reactions were monitored by TLC and LC-MS analysis.

Entry	Starting material	Reagents	Solvent	T (°C)	t (h)	Results
1		DPPA (1 eq.) Et_3N (1.4 eq.)	CH_2Cl_2	r.t.	1	No reaction
				50	1	No reaction
				75	12	Starting materials consumed, formation of complex RM
		PMB-NH_2 (2 eq.)		r.t.	1	No target detected, complex RM
2		DPPA (1 eq.)	Pyridine	r.t.	1	No reaction
				50	1	No reaction
				75	12	Starting materials consumed, formation of complex RM
		PMB-NH_2 (2 eq.)		r.t.	1	No target detected, complex RM
3		DPPA (1 eq.) Et_3N (1.4 eq.)	Toluene	r.t.	1	Full conversion of starting materials
				75	12	
		PMB-NH_2 (2 eq.)		r.t.	1	Successful reaction
4		DPPA (1 eq.) Et_3N (1.4 eq.)	CH_3CN	r.t.	1	Full conversion of starting materials
5		DPPA (1 eq.) Et_3N (1.4 eq.)	CH_3CN	r.t.	1	No reaction
					4	No reaction
		BH_3-THF (1 eq.)		75	2	Starting materials consumed, formation of complex RM
		PMB-NH_2 (2 eq.)		r.t.	12	No target detected, complex RM

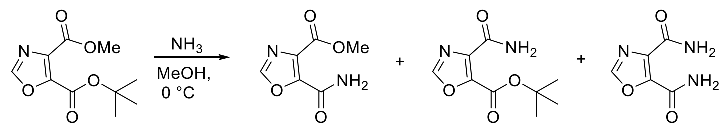
From the experiments which have been described in Table 10, it was not clear if 4-(methoxycarbonyl)oxazole-5-carboxylic acid reacted with diphenyl phosphoryl azide at 75 °C to give the intermediate acyl azide or not. Therefore, the synthesis of the acyl azide was attempted *via* the classic two-step pathway, *i.e.* conversion of the carboxylic acid to the acyl chloride, followed by substitution of the chloride by reaction with sodium azide.[157] When oxazole **342** was treated with a small excess of oxalyl chloride and a catalytic amount of DMF at room temperature for one hour, the acyl chloride **348** was obtained (Scheme 77). TLC and ^{1}H- and ^{13}C-NMR analysis proved a successful reaction, and the crude acyl chloride was used for the next step. The crude acyl chloride **348** was then reacted with aqueous sodium azide in acetone at 0 °C for one hour, giving the crude acyl azide **349** in 86% yield after aqueous work-up. Again, the crude product was used for the next step, after TLC and ^{1}H- and ^{13}C-NMR analysis showed that the acyl azide was formed in high purity. The crude acyl azide was dissolved in dry toluene and heated at 75 °C. TLC analysis showed full conversion of the azide after two hours. Several new spots were observed on TLC, but this could be due to reaction with atmospheric moisture or instability of the azide on silica. The reaction was cooled down and two equivalents of either 3,4,5-trimethoxybenzylamine or 2,4,5-trimethoxybenzylamine were added to the reaction. LC-MS analysis showed formation of a complex reaction mixture in both cases. Only a trace of the desired ureids **350** could be detected.



Scheme 77. The Curtius rearrangement of 4-(methoxycarbonyl)oxazole-5-carboxylic acid was not successful

2.3 Synthesis of the monocarboxamide precursors for the Hofmann rearrangement reaction

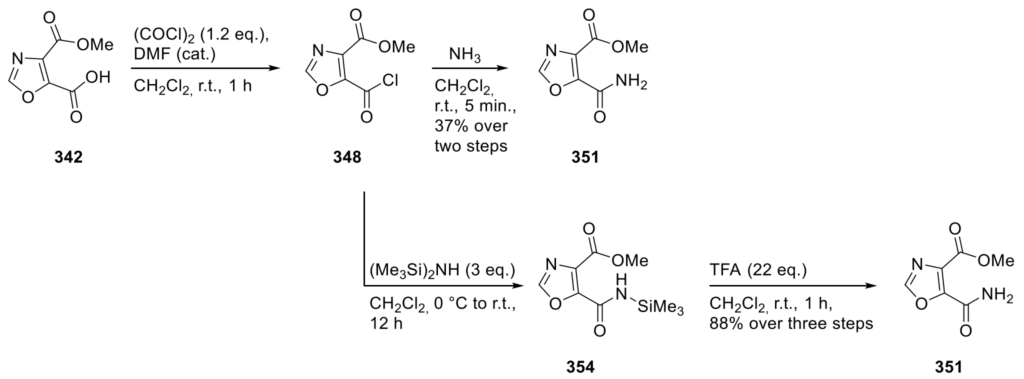
A Hofmann rearrangement, induced by a hypervalent iodine reagent, was chosen to replace the unsuccessful Curtius rearrangement for two reasons. First, this type of Hofmann rearrangement can be performed at room temperature. Secondly, the reaction typically does not require addition of a base.[158–160] These mild conditions could avoid the occurrence of the undesired side reactions which were observed during the Curtius rearrangement. However, the Hofmann rearrangement converts a primary amide and not a carboxylic acid group to an isocyanate. Therefore, the primary amides had to be synthesized first. Aminolysis of 5-(*tert*-butyl) 4-methyl oxazole-4,5-dicarboxylate **340c** with ammonia in methanol at 0 °C resulted in formation of both amides **351** and **352** and the diamide **353**, as expected (Scheme 78). Using one equivalent of ammonia, no reaction was observed after one hour at 0 °C. Then, a large excess (approximately 30 equivalents) of ammonia was added. After 40 minutes at 0 °C, mainly starting material was present, next to both mono-amides and the diamide.



340c 351 352 353

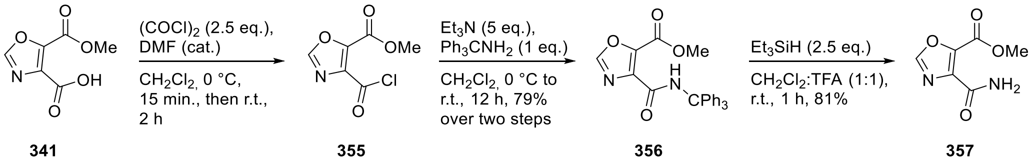
Scheme 78. Aminolysis of 5-(tert-butyl) 4-methyl oxazole-4,5-dicarboxylate did not proceed chemoselectively

Reaction of the acyl chloride **348** with ammonia in dichloromethane gave the desired amide **351** in 37% yield over two steps, next to unidentified side products (Scheme 79). Formation of the diamide was not observed. Pellegata and coworkers reported a mild method to convert acyl chlorides to primary carboxamides by using hexamethyldisilazane as a synthetic equivalent of ammonia.[161] Treatment of the acyl chloride **348** with three equivalents of hexamethyldisilazane in dichloromethane gave the crude mono-trimethylsilyl protected amide **354**, which was subsequently fully deprotected by treatment with trifluoroacetic acid in dichloromethane. The primary amide **351** was obtained in 88% yield over three steps.



*Scheme 79. Synthesis of methyl 5-carbamoyloxazole-4-carboxylate **351***

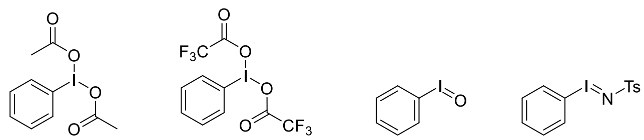
The synthesis of methyl 4-carbamoyloxazole-5-carboxylate was attempted following the same method as described above. Remarkably, hexamethyldisilazane failed to react with the acyl chloride. Theodorou and coworkers described a method to prepare primary amides from acyl chlorides by reaction with tritylamine as a synthetic equivalent of ammonia.[162] Treatment of 5-(methoxycarbonyl)oxazole-4-carboxylic acid **341** with an excess of oxalyl chloride and a catalytic amount of DMF afforded the crude acyl chloride **355** (Scheme 80). Reaction of this acyl chloride with one equivalent of tritylamine and an excess of triethylamine afforded the trityl-protected amide **356** in 79% yield over two steps. Deprotection of the amide using triethylsilane as a cation scavenger in a mixture of TFA and dichloromethane gave methyl 4-carbamoyloxazole-5-carboxylate **357** in 81% yield.



*Scheme 80. Synthesis of methyl 4-carbamoyloxazole-5-carboxylate **357***

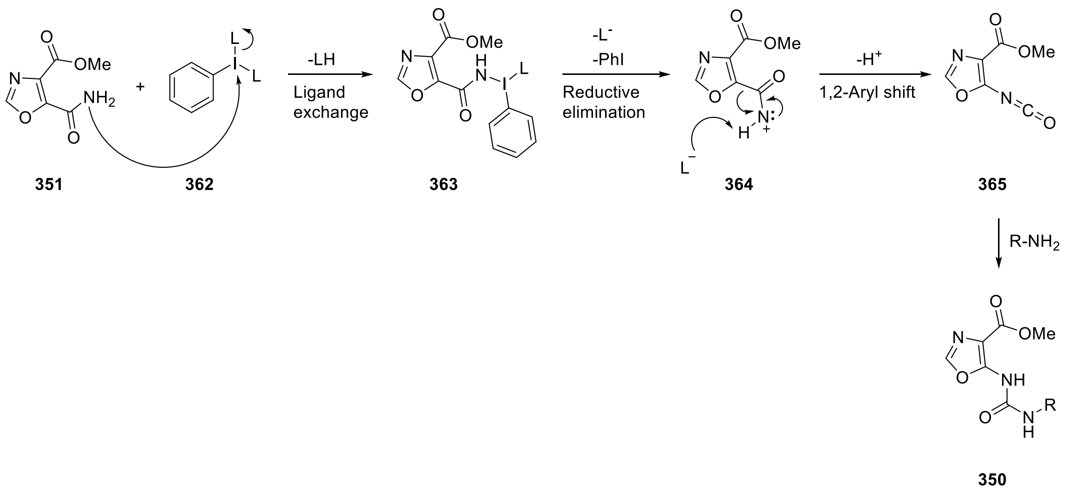
2.4 Evaluation of the Hofmann rearrangement reaction towards oxazolo[5,4-*d*]pyrimidines

With the primary amides in hand, the hypervalent iodine-mediated Hofmann rearrangement could now be investigated. The most commonly employed hypervalent iodine reagents are the organoiodine(III) compounds (diacetoxyiodo)benzene [PhI(OAc)$_2$] **358**, [bis(trifluoroacetoxy)iodo]benzene [PhI(OCOCF$_3$)$_2$] **359**, iodosylbenzene (PhIO) **360** and (tosylimino)phenyl-λ^3-iodane (PhINTs) **361** (Figure 16).[160] The proposed reaction mechanism is depicted in Scheme 81. The primary carboxamide **351** first substitutes one of the ligands of the hypervalent iodine reagent, to give the intermediate *N*-(phenyl-λ^3-iodanyl)carboxamide **363**. The following step is a reductive elimination of iodobenzene, resulting in the nitrenium species **364**. This reactive intermediate then undergoes a 1,2-aryl shift to afford the isocyanate **365**, which can be treated with an appropriate amine to give the final ureids **350**. Whether the nitrenium species is actually formed or the reductive elimination and 1,2-aryl shift proceed in a concerted fashion remains a matter of debate.[158,163–167]



358 **359** **360** **361**

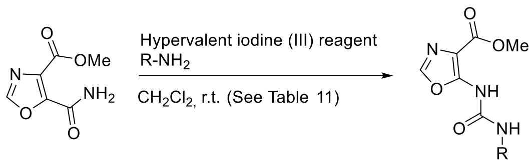
Figure 16. Typical hypervalent iodine reagents used in the Hofmann rearrangement



*Scheme 81. Proposed mechanism of the transformation of primary carboxamide **351** to its corresponding N-3 substituted ureid **350***

An initial qualitative screening was performed, using different amines and hypervalent iodine reagents (Scheme 82, Table 11). Reaction of carboxamide **351** with 1.5 equivalents of propylamine and 1.5 equivalents of iodosylbenzene for two hours at room temperature gave a relatively clean conversion to the desired ureid **350** (entry 1). Under the same reaction conditions, the more sterically hindered cyclohexylamine gave nearly identical results (entry 2). When benzylamine was used under these conditions, unreacted starting material **351** was present next to the ureid **350** in a one-to-one ratio (entry 3). One of the observed side products was imine **366**, resulting from oxidation of benzylamine (Figure 17). The oxidation of amines with hypervalent iodine reagents has been described in the literature.[168–170] However, the release of water by iodosylbenzene during the Hofmann rearrangement requires the concurrent presence of the iodine reagent and the amine to avoid addition of water to the isocyanate. When 3,4,5-trimethoxybenzylamine was used (entry 4), a large peak of imine **367** (Figure 17) was detected, and formation of the desired ureid was not observed. The use of PhINTs was reported by Yoshimura and coworkers as a mild reagent which allowed the transformation of substituted benzamides to their respective isocyanates.[171] Unfortunately, the use of this iodine reagent resulted only in the formation of many side products, and the desired ureid could not be detected (entry 5). When (diacetoxyiodo)benzene was reacted with carboxamide for two hours, followed by addition of benzylamine, a minor amount of starting material was observed in the reaction mixture after one hour, next to a major amount of the desired ureid and different side products (entry 6). Stirring the reaction further overnight did not result in any significant changes of the reaction mixture. When [bis(trifluoroacetoxy)iodo]benzene was used under the same reaction conditions, a large amount of unreacted starting material remained in the reaction mixture, next to different side products (entry 7). Formation of ureid **350** was not observed.

Based on this initial screening, iodosylbenzene proved a suitable reagent for the Hofmann rearrangement in the presence of amines that are relatively inactive towards oxidation. The use of (tosylimino)phenyl-λ^3-iodane or [bis(trifluoroacetoxy)iodo]benzene did not give the desired ureid and these reagents were abandoned from further study. The use of (diacetoxyiodo)benzene looked promising but still required reaction optimization.



351 **350**

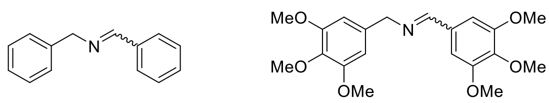
*Scheme 82. Transformation of carboxamide **351** to ureid **350** via a Hofmann rearrangement*

Table 11. Initial screening of the hypervalent iodine-mediated Hofmann rearrangement

Entry	Reagent (eq.)	Amine (eq.)	t (h)	Results
1	PhIO (1.5)	*n*-Pr-NH_2 (1.5)	2	**350** (major) + side products (minor)
2	PhIO (1.5)	*c*-Hex-NH_2 (1.5)	2	**350** (major) + side products (minor)
3	PhIO (1.5)	Bn-NH_2 (1.5)	2	**351**:**350** (1:1) + side products
4	PhIO (1.5)	3,4,5-$(MeO)_3$-$C_6H_2CH_2NH_2$ (1.5)	2	Conversion to side products, no **350** detected
5	PhINTs (1.2)	*n*-Pr-NH_2 (1.5)[a]	0.5	**351** (minor) + side products (major), no **350** detected
6	$PhI(OAc)_2$ (1.5)	Bn-NH_2 (1.5)[b]	1	**351** (minor) + **350** (major) + side products (major)

7	$PhI(OCOCF_3)_2$ (1.5)	$Bn\text{-}NH_2$ (1.5)[b]	1	**351** (major) + side products (minor), no **350** detected

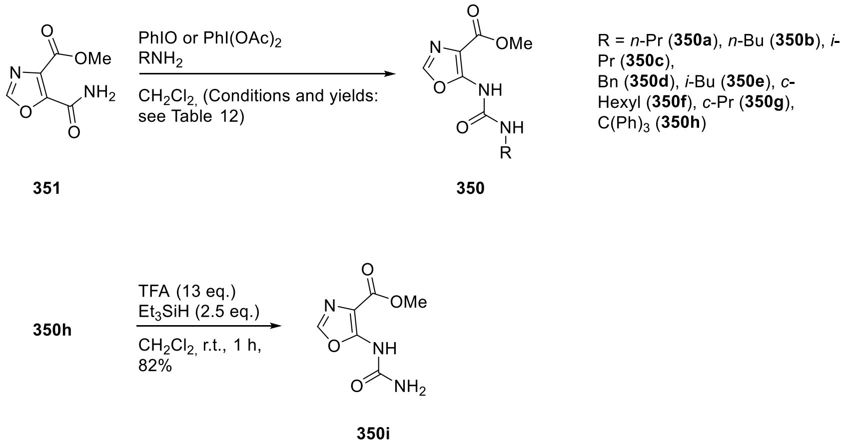
[a/b]: amine was added after 1.5 h[a] or 2 h[b] of reaction time between the carboxamide and the iodine reagent.



366 **367**

Figure 17. Side products observed during the Hofmann rearrangement experiments

Next, iodosylbenzene and (diacetoxyiodo)benzene were used to construct a small series of ureids **350** (Scheme 83). For (diacetoxyiodo)benzene, a small optimization study was done with isopropylamine and the following reaction conditions were found to give the most satisfactory results: reacting carboxamide **351** with three equivalents of (diacetoxyiodo)benzene in dichloromethane for two hours at room temperature, followed by addition of six equivalents of isopropylamine and continued stirring for twelve hours. For *n*-propyl, *n*-butyl and isopropyl, the use of (diacetoxyiodo)benzene gave similar results as the use of iodosylbenzene, and the desired ureids **350a-c** were obtained in 52-65% yield (Table 12, entry 1-6). For benzyl derivative **350d**, the amount of both iodosylbenzene and benzylamine was increased to three equivalents, in an attempt to increase the conversion of the carboxamide **351** (entry 7). Unfortunately, the conversion of starting material was incomplete and **351** was still present in a 3:7 ratio to the ureid **350d**, next to various side products. This resulted in a low isolated yield of 18%. The reaction with (diacetoxyiodo)benzene was adjusted as well (entry 8). First, the carboxamide **351** was refluxed with six equivalents of (diacetoxyiodo)benzene for two and a half hours to convert all of the carboxamide to the isocyanate. Then, the mixture was cooled down to 0 °C and six equivalents of benzylamine were added. The reaction was allowed to warm up to room temperature and continued stirring for twelve hours. After purification, the ureid **350d** was obtained in 15% yield. The low yield could be explained by (oxidative) side reactions. Based on these reactions, the use of iodosylbenzene did not give a clear advantage over the use of (diacetoxyiodo)benzene, and since it is a potentially explosive compound, (diacetoxyiodo)benzene was preferred for further reactions.[172] The isobutyl and cyclohexyl derivatives **350e** and **350f** were synthesized using (diacetoxyiodo)benzene and were obtained in 51 and 63% yield, respectively (entry 9-10). The cyclopropyl ureid **350g** was isolated in only 19% yield after a difficult purification (entry 11). When *tert*-butylamine or tritylamine were used under the typical reaction conditions for (diacetoxyiodo)benzene (*cfr*. entry 2), oxidative degradation of the ureid product by the excess amount of oxidant was observed. This degradation process could be halted by adding a quenching agent or a sacrificial agent such as isopropylamine to the reaction. For tritylamine, the same reaction conditions were used as for benzylamine, except that the reaction was kept at 0 °C after addition of tritylamine and quenched by aqueous sodium thiosulfate after half an hour (entry 12). Despite these efforts, a significant amount of side products were formed and the desired ureid **350h** was isolated in 31% yield. Compound **350h** could be deprotected by treatment with trifluoroacetic acid and triethylsilane, affording ureid **350i** in 82% yield.



*Scheme 83. Synthesis of ureids **350** via a Hofmann rearrangement on carboxamides **351**, and deprotection of **350h** into **350i***

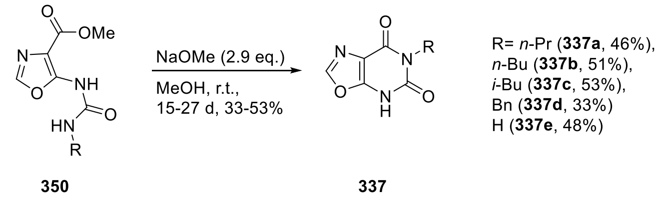
*Table 12. Synthesis of ureid compounds **350** via a Hofmann rearrangement on carboxamide **351**. All reactions were conducted in dichloromethane and at room temperature, unless mentioned otherwise*

Entry	Reagent	(eq.)	Amine	(eq.)	Addition of amine	t (h)	Yield (%)
1	PhIO	1.5	*n*-Pr-NH_2	1.5	At start	2	63
2	$PhI(OAc)_2$	3	*n*-Pr-NH_2	6	After 2 h, at r.t.	12	58
3	PhIO	1.5	*n*-Bu-NH_2	1.5	At start	12	56
4	$PhI(OAc)_2$	3	*n*-Bu-NH_2	6	After 2 h, at r.t.	12	56
5	PhIO	1.5	*i*-Pr-NH_2	1.5	At start	12	52
6	$PhI(OAc)_2$	3	*i*-Pr-NH_2	6	After 2 h, at r.t.	1	65
7	PhIO	3	Bn-NH_2	3	At start	12	18
8	$PhI(OAc)_2$	6	Bn-NH_2	6	After 2.5h reflux, at 0 °C	12	15
9	$PhI(OAc)_2$	3	*i*-Bu-NH_2	6	After 2 h, at r.t.	12	51
10	$PhI(OAc)_2$	3	*c*-Hexyl-NH_2	6	After 2 h, at r.t.	12	63
11	$PhI(OAc)_2$	3	*c*-Pr-NH_2	6	After 2 h, at r.t.	12	19
12	$PhI(OAc)_2$	6	$(Ph)_3CNH_2$	6	After 2.5h reflux, at 0 °C	0.5[a]	31

[a]: Reaction at 0 °C, followed by sat. aq. $NaHSO_3$ quench.

2.5 Synthesis of oxazolo[5,4-*d*]pyrimidines via ring closure of the ureid precursors

The final step, ring closure of the ureids **350** to the oxazolo[5,4-*d*]pyrimidine-5,7-diones **337**, was effectuated by reaction with 2.9 equivalents of sodium methoxide in methanol at room temperature (Scheme 84). The reaction proceeded very slowly, and full conversion was observed after fifteen (**337e**) or twenty-seven (**337a-d**) days of stirring at room temperature. The oxazolo[5,4-*d*]pyrimidine-5,7-diones were isolated in 33-53% yield. The low to moderate yields can be explained by formation of side products, and losses during isolation of the products. The ureids bearing an isopropyl, cyclohexyl, cyclopropyl or trityl group failed to ring-close under these reaction conditions, most likely due to steric hindrance. When the temperature of this reaction was raised to 50 °C, more side products were formed in general, and no ring closure was observed for the sterically hindered derivatives.



Scheme 84. Ring closure of ureid structures **350** afforded oxazolo[5,4-d]pyrimidine-5,7-diones **337**

Other reaction conditions were screened in order to improve the ring closure reaction of ureids **350** (Table 13). The use of lithium hexamethyldisilazide resulted in the formation of side products, and the desired ring structure was not detected (entry 1). The use of potassium *tert*-butoxide could not induce the ring closure either (entry 2). Next to the remaining starting material, only side products were formed. Sodium hydride did not give any conversion in tetrahydrofuran as a solvent (entry 3). When *N*,*N*-dimethylformamide was added to the reaction, the ureid started to convert only to side products. When the ureid was heated in the microwave reactor in acetonitrile at 100 °C for twelve hours, no conversion was observed (entry 4). Heating the ureid in DMF at 160 °C for fifteen minutes in the microwave reactor resulted in side product formation (entry 5). Treatment with two and a half equivalents of lithium diisopropylamide at – 78 °C did not give any conversion at first, but when the reaction was allowed to warm up to room temperature, full conversion to side products occurred (entry 6). An attempt was made to achieve ring closure with a catalytic amount of sodium methoxide in methanol (entry 7). After two hours at 70 °C in the microwave reactor, mainly starting material was present, next to the desired ring structure and side products. The experiment was not continued due to the increased formation of side products compared to the reaction at room temperature. Next, the sodium methoxide-methanol system was evaluated for ring closure of the sterically hindered isopropylureid **350c**. Treatment with one, two or four equivalents of sodium methoxide in methanol at 70 °C for one hour and fifteen minutes resulted in an incomplete conversion to side products and a trace amount of the desired ring structure **337** (entry 8-10). The use of potassium *tert*-butoxide in *tert*-butanol gave inferior results compared to the sodium methoxide-methanol system, as no formation of the ring structure was observed (entry 11). In DMSO as a solvent, a large amount of side products was formed after half an hour at 85 °C in the microwave reactor, without formation of the ring structure (entry 12). In DMF as a solvent, not much conversion was observed under these reaction conditions (entry 13). Finally, some acid-catalyzed reaction conditions were tested.[173,174] Heating of the ureid in concentrated hydrochloric acid at 100 °C for two minutes gave full conversion to (chlorine-containing) side products (entry 14). In a mixture of ethanol and aqueous concentrated hydrochloric acid, complete conversion to (chlorine-containing) side products was seen after half an hour at 100 °C in a microwave reactor (entry 15).

Table 13. Reaction conditions screening for the ring closure of ureids **350**

Entry	Ureid	Reagent (eq.)	Solvent	T (°C)	t (h)	Results
1	**350a** (R = *n*-Pr)	LiHMDS (1.1)	THF	0	1	Starting material + side products
		(+ 1.1)		r.t.	1.5	Full conversion to side

						products
2		KO*t*-Bu (1.1)	THF	0	1	Starting material + side products
		(+ 1.1)		r.t.	12	Starting material + side products
3		NaH (1.1)	THF	0	1	No conversion
		(+ 1.1)		r.t.	12	No conversion
			+ DMF (THF:DMF, 1:2)	r.t.	12	Starting material + side products
4		No reagent	CH_3CN	100 (µW)	12	No conversion
5		No reagent	DMF	160	0.25	Starting material + side products
6		LDA (2.5)	THF	-78	0.5	No conversion
				r.t.	1	Full conversion to side products
7	**350d** (R = Bn)	NaOMe (0.1)	MeOH	70 (µW)	2	**350d** (major) + **337d** (minor) + side products (minor)
8	**350c** (R = *i*-Pr)	NaOMe (1)	MeOH	70	1.25	Starting material (major) + side products (minor) + **337** (trace)
9		NaOMe (2)	MeOH	70	1.25	Starting material (major) + side products (minor) + **337** (trace)
10		NaOMe (4)	MeOH	70	1.25	Starting material (major) + side products (minor) + **337** (trace)
11		KO*t*-Bu (1)	*tert*-BuOH	70	1.25	Starting material + side products
12		NaOMe (1)	DMSO	85 (µW)	0.5	Starting material + side products
13		NaOMe	DMF	85 (µW)	0.5	No conversion
		(+ 1)		85 (µW)	1	No conversion
14		Conc. HCl (15)	Neat	100	2 min.	Conversion to side products
15		Conc. HCl (15)	EtOH	100 (µW)	0.5	Conversion to side products

Based on this screening of reaction conditions and on the preceding ring closure experiments, it was concluded that the use of sodium methoxide in methanol at room temperature was the best system to ring-close the ureids **350** to the oxazolo[5,4-*d*]pyrimidine-5,7-diones **337**, although it was characterized by very long reaction times. Heating of the reaction mixture could increase the reaction speed, but also gave rise to the formation of side products which greatly impeded the purification of the final ring closed compounds.

When side products were formed during the ring closure attempts of *n*-propylureid **350a** and isopropylureid **350c**, very frequently an LC-MS peak with the same m/z value as the ureid starting material was detected among the side product peaks. Intrigued by this particular side product, it was isolated from one of the experiments and characterized by ^{1}H- and ^{13}C-NMR spectrometry. The NMR data suggested the isoureid structure **368** (Figure 18, left): one of the N-H proton signals has disappeared, while the remaining N-H proton couples with the C-H proton of the isopropyl group. Normally, the isoureid tautomer is less stable than the ureid form, but the possibility of intramolecular hydrogen bonding between the oxazole oxygen atom and the isoureid hydroxyl group could explain the stability of the isoureid (Figure 18, right).[175]

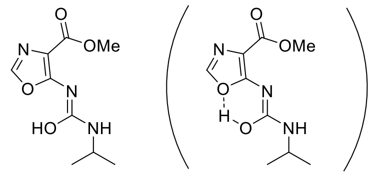


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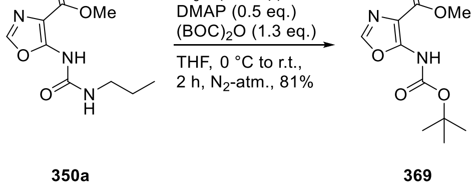
Figure 18. Suggested structure of an isolated side product after the attempted ring closure of isopropylureid (left) and possible intramolecular hydrogen bonding of this structure (right)

An attempt was made to protect the nitrogen atom which is directly connected to the oxazole ring with a BOC protecting group. After BOC protection, the effect on the ring closure could then be investigated. However, treatment of the ureid **350a** with 1.3 equivalents of both triethylamine and di-*tert*-butyldicarbonate and a catalytic amount of DMAP unexpectedly afforded the BOC protected amine **369** in 81% yield (Scheme 85).[176]



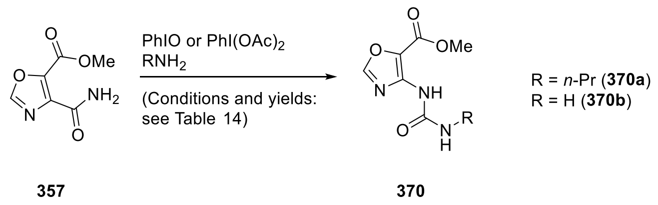
350a **369**

*Scheme 85. DMAP-catalyzed reaction of ureid **350a** with di-tert-butyldicarbonate afforded BOC-protected amine **369***

2.6 Evaluation of the Hofmann and Curtius rearrangement reaction towards oxazolo[4,5-*d*]pyrimidines

The Hofmann rearrangement was also performed on methyl 4-carbamoyloxazole-5-carboxylate **357** in pursuit of the oxazolo[4,5-*d*]pyrimidine-5,7-dione isomers (Scheme 86, Table 14). Only the *n*-

propylureid **370a** was constructed due to time restraints. In this case, iodosylbenzene performed better than (diacetoxyiodo)benzene, and the ureid **370a** was obtained in 85% yield and 61% yield, respectively. When the Hofmann rearrangement reaction was performed in a solution of 7M ammonia in methanol instead of dichloromethane, formation of the diamide was observed next to other minor side products (Table 14, entry 3 and 4). The desired ureid **370b** was not formed in these reactions. Addition of another 1.5 equivalents of (diacetoxyiodo)benzene to the reaction (entry 4, after five hours) in an attempt to cyclize the diamide, did not result in formation of the ring-closed product.



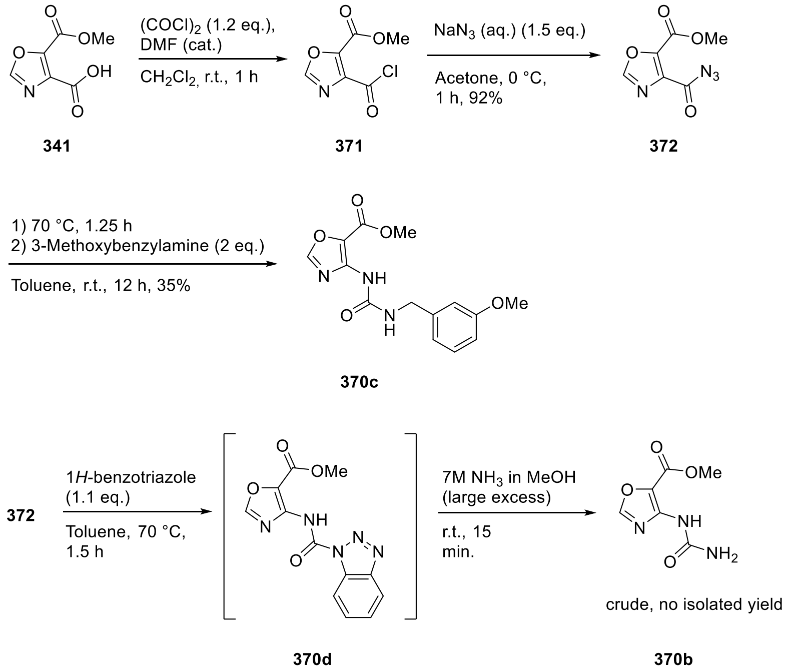
*Scheme 86. Synthesis of ureid **370** via a Hofmann rearrangement on methyl 5-carbamoyloxazole-4-carboxylate*

*Table 14. Reaction conditions and yields for the Hofmann rearrangement on carboxamide **357***

Entry	Reagent	(eq.)	RNH_2 (eq.)	Addition of amine	t (h)	Yield (%)
1[a]	PhIO	1.5	*n*-$PrNH_2$ (1.5)	At start	2	85
2[a]	$PhI(OAc)_2$	3	*n*-$PrNH_2$ (6)	After 2 h, at r.t.	0.5	61
3[b]	PhIO	1.5	NH_3 (60)	At start	5	/
4[b]	$PhI(OAc)_2$	1.5	NH_3 (60)	At start	5	/

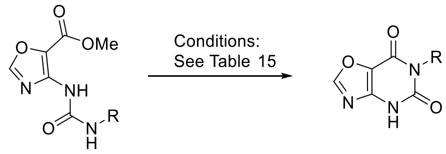
[a]: Reaction in dichloromethane. [b]: Reaction in methanol.

The Curtius rearrangement was unsuccessful on 4-(methoxycarbonyl)oxazole-5-carboxylic acid (*vide supra*), but was still evaluated for its isomer 5-(methoxycarbonyl)oxazole-4-carboxylic acid, following the same synthetic protocol. Thus, carboxylic acid **341** was treated with a small excess of oxalyl chloride and a catalytic amount of DMF in dichloromethane at room temperature for one hour, affording the acyl chloride **371** (Scheme 87). The crude acyl chloride was then substituted by reaction with sodium azide, giving the acyl azide **372** in 92% yield over two steps. When this acyl azide was heated at 70 °C for one hour and fifteen minutes, TLC analysis showed complete conversion of the starting material. The reaction was cooled down to room temperature, and two equivalents of 3-methoxybenzylamine were added. After twelve hours, LC-MS analysis of the reaction mixture showed that the desired ureid **370c** was formed -next to a significant amount of side products- and was isolated in 35% yield. For the synthesis of ureid **370b**, another approach was used in an attempt to increase the yield of the reaction. Carboxylic acid **341** and 1.1 equivalents of 1*H*-benzotriazole were suspended in dry toluene and heated to 70 °C. After a few minutes, the suspension turned into a clear solution and gas formation was observed. After 1.5 hours, the formation of gas had ceased and the solvent was evaporated to afford the crude ureid **370d** as an off-white solid. The crude material was dissolved in a solution of 7M of ammonia in methanol (large excess of ammonia) and stirred at room temperature for fifteen minutes. Full conversion of **370d** was observed, but product **370b** could not be isolated and still contained residual 1*H*-benzotriazole a small amount of side products. The crude product was used for further reaction.



*Scheme 87. Synthesis of ureids **370c** and **370b** via a Curtius rearrangement*

Different attempts were made to ring-close the ureids to their corresponding oxazolo[4,5-*d*]pyrimidine-5,7-dione counterparts (Scheme 88). Ring closure of the ureids was attempted using the sodium methoxide-methanol system (Table 15, entry 1-2). Unfortunately, the reaction of **370** with 2.9 equivalents of sodium methoxide in methanol afforded only side products after twelve hours of stirring (R = *n*-Pr) or sixty hours of stirring (R = 3-methoxybenzyl). When 1.1 equivalents of potassium *tert*-butoxide were used as a base in tetrahydrofuran, no reaction occurred (entry 3). After an additional 1.1 equivalents of potassium *tert*-butoxide were added and stirring continued for three days, small amounts of side products started to appear next to the unreacted starting material. Only traces of the desired ring-closed product could be detected. Subsequently, 1.1 equivalents of potassium hexamethyldisilazide (KHMDS) were added and stirring continued for one hour at room temperature. This resulted in an increase of the amount of side products, while unreacted starting material was still present. Finally, the use of one equivalent of sodium hydroxide (1M in H_2O) in ethanol at 80 °C for two hours gave the desired ring closed product **333a** in 6% yield (over three steps, from **372** to **333a**).[177] Significant improvements of the yield of this reaction may be possible on a larger scale and after optimization of the reaction conditions and the work-up protocol. Ureids **370a** and **370c** could not be obtained anymore with this method due to time constraints, but a trial with ureid **370a** showed a large peak of the desired ring closed product in LC-MS, indicating that this ring closure protocol also works for this *n*-propylureid.



R = H (**333a**)

370 **333**

*Scheme 88. Ring closure of ureids **370** towards the oxazolo[4,5-d]pyrimidine-5,7-diones **333***

*Table 15. Attempts to ring-close the ureids **370** to the oxazolo[4,5-d]pyrimidine-5,7-diones **333***

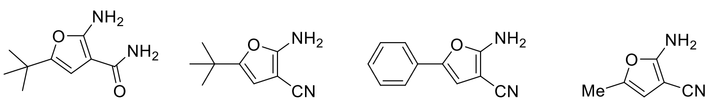
Entry	Ureid	Reagent (eq.)	Solvent	T (°C)	t (h)	Results
1	**370a** (R = *n*-Pr)	NaOMe (2.9)	MeOH	r.t.	12	Full conversion to side products
2	**370c** (R = 3-MeO-$C_6H_4CH_2$)	NaOMe (2.9)	MeOH	r.t.	12	Starting material + side products
					60	Full conversion to side products
3	**370a**	KO*t*-Bu (1.1)	THF	0 °C	0.25	No conversion
				0 °C	2	No conversion
				r.t.	2.5	No conversion
		(+1.1)		r.t.	72	Starting material + side products
		+ KHMDS (1.1)		r.t.	1	Starting material + side products
4	**370b**	NaOH (1M in H_2O, 1 eq.)	EtOH	80	2	**333a**, 6% isolated yield (from **372** to **333a**)

2.7 Conclusions

In conclusion, an efficient two-step pathway was developed towards 4-(methoxycarbonyl)oxazole-5-carboxylic acid and 5-(methoxycarbonyl)oxazole-4-carboxylic acid. The conversion of these carboxylic acids to their corresponding ureids *via* a Curtius rearrangement was only achieved in the case of 5-(methoxycarbonyl)oxazole-4-carboxylic acid, although in low yield. Both carboxylic acids were further transformed to their corresponding primary amides, methyl 5-carbamoyloxazole-4-carboxylate and methyl 4-carbamoyloxazole-5-carboxylate, *via* the intermediate acyl chlorides. A hypervalent iodine-mediated Hofmann rearrangement on the primary amides then afforded the corresponding *N*-3 substituted ureids in varying yields. The combination of oxidation-sensitive amines and the hypervalent iodine oxidant proved problematic, limiting the scope of this reaction. The oxazolo[5,4-*d*]pyrimidine-5,7-dione scaffold and a handful of *N*-3 substituted derivatives could be obtained in low to moderate yields via ring closure of the corresponding ureids by treatment with sodium methoxide in methanol, except for sterically hindered ureids. The oxazolo[4,5-*d*]pyrimidine-5,7-dione scaffold could be obtained via ring closure of the corresponding ureid by treatment with aqueous sodium hydroxide in ethanol at elevated temperature, although in very low yield.

3 Synthesis of 2-amino-(5-*tert*-butyl)furan-3-carboxamide

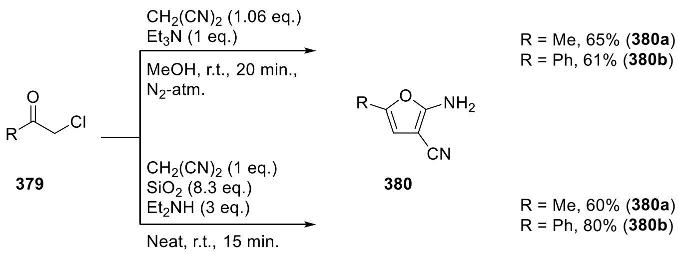
A pharmaceutical company requested the synthesis of a specific molecule to include it in one of their lead optimization campaigns. This molecule was 2-amino-(5-*tert*-butyl)furan-3-carboxamide **375** (Figure 19). A literature search revealed that this compound had not been reported yet. A similarity search in the literature then gave the 3-carbonitrile analogue **376** as a closely related structure and possible precursor for the synthesis of **375**.[178–182] Unfortunately, none of the literature references described the synthesis of compound **376**. The similarity search was extended and structures **377** and **378**, equipped with a phenyl and methyl substituent at the 5-position respectively, were found.[183,184]



375 **376** **377** **378**

*Figure 19. Target structure **375** and related molecules which were found in the literature*

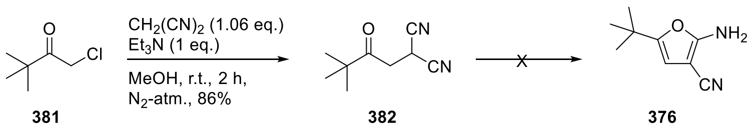
According to Jun, reaction of α-chloroketone **379** with a slight excess of malononitrile and one equivalent of triethylamine in methanol afforded the 5-substituted-2-aminofuran-3-carbonitriles **380** in 61-65% yield (Scheme 89, top).[183] Bakavoli and coworkers used solvent-free conditions to synthesize the same structures. According to their work, grinding a mixture of α-chloroketone **379**, one equivalent of malononitrile, three equivalents of diethylamine and an excess of silica in a mortar for 15 minutes afforded the desired products **380** in 60-80% yield (Scheme 89, bottom).[184]



Scheme 89. Synthesis of 5-substituted-2-aminofuran-3-carbonitriles according to Jun (top) and Bakavoli and coworkers (bottom)

3.1 Synthesis of 2-amino-5-(*tert*-butyl)furan-3-carbonitrile

When 1-chloropinacolone was reacted with malononitrile under the reaction conditions of Jun, complete conversion of starting material was observed after two hours, and LC-MS analysis indicated that a new product with the correct target mass was formed. However, after isolation of this product in 86% yield, NMR analysis showed that the substitution product **382** was formed and that it had not ring closed to the desired 2-amino-5-(*tert*-butyl)furan-3-carbonitrile **376**, which is an isomer of **382** (Scheme 90). The reaction conditions of Bakavoli *et al.* were also evaluated for the reaction of 1-chloropinacolone with malononitrile. Under these conditions, different side products were formed and the substitution product **382** was isolated in only 7% yield. Formation of the desired 2-amino-5-(*tert*-butyl)furan-3-carbonitrile **376** could not be detected. When 1-chloropinacolone was reacted with one equivalent of malononitrile and one equivalent of diethylamine in tetrahydrofuran as a solvent, most of the 1-chloropinacolone was attacked by diethylamine instead of malononitrile, resulting in a huge amount of undesired side product. Hence, the use of diethylamine should be avoided for this reaction and it should be replaced by the non-nucleophilic base triethylamine.



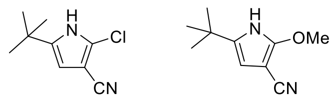
Scheme 90. Reaction of 1-chloropinacolone with malononitrile

The next step was a screening of reaction conditions for the ring closure of **382** to **376** (Table 16). Treatment with potassium *tert*-butoxide or sodium hydride in THF resulted in complex reaction mixtures (entries 1-4). Nakano *et al.* have reported acidic reaction conditions to affect a similar ring closure with success.[185] When three equivalents of aqueous hydrochloric acid in acetic acid were used (entry 5), different side products were formed. The proposed structure of the major side product (**383**) is depicted in Figure 20. Bakavoli *et al.* reported the use of neat TFA to accomplish a similar ring closure.[184] Unfortunately, the use of neat TFA lead to a complex reaction mixture (entry 6). The reaction conditions of Matsuda *et al.*,[186] using piperidine in ethanol at reflux temperature (entry 7), and the conditions reported by Nakano *et al.*,[185] using piperazine in ethanol at room temperature (entry 9) both resulted in complex reaction mixtures. Switching to triethylamine as a base in ethanol (entry 8) or water (entry 10) or diethylamine in water (entry 11) still resulted in complex reaction mixtures. When triethylamine was used in methanol at 90 °C under microwave irradiation (entry 12), a complex reaction mixture was obtained again, and methoxypyrrole structure **384** (Figure 20) was proposed as a major side product. Based on the suspected formation of the undesired pyrroles **383** and **384** during this reaction screening, it was proposed that the presence of nucleophiles should be avoided during the ring closure reaction. Changing the solvent to toluene and heating of the reaction mixture for 10 minutes at 110 °C under microwave irradiation lead to a complex reaction mixture again (entry 13). The use of THF as a solvent and triethylamine as a base at 65 °C under microwave irradiation for thirty minutes gave 15% conversion of the starting material to the desired product **376** (entry 14). The amount of triethylamine was increased to a 1:3 v/v ratio to THF and the reaction temperature was raised to 90 °C in order to speed up the reaction (entry 15). After one hour, full conversion was observed and 2-amino-5-(*tert*-butyl)furan-3-carbonitrile **376** was isolated in 85% yield.

*Table 16. Screening of reaction conditions for the ring closure of **382.** 1.06 equivalents of malononitrile were used in all experiments.*

Entry	Solvent	Reagent	T (°C)	t (h)	Results
1	THF	KO*t*Bu (1 eq.)	r.t.	0.5	Complex RM
2	THF	NaH (1 eq.)	r.t.	0.5	Complex RM
3	THF	KO*t*Bu (2 eq.)	r.t.	0.5	Complex RM
4	THF	NaH (2 eq.)	r.t.	0.5	Complex RM
5	CH_3COOH	Aq. HCl (3 eq.)	r.t.	4	Complex RM
6	TFA	/	r.t.	1	Complex RM
7	EtOH	Piperidine (2.5 eq.)	Reflux	1	Complex RM

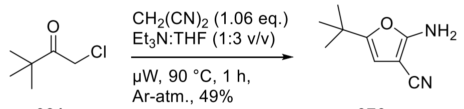
8	EtOH	Et_3N (1:3 v/v to EtOH)	Reflux	1	Complex RM
9	EtOH	Piperazine (2.5 eq.)	r.t.	1	Complex RM
10	H_2O	Et_3N (1:2 v/v to H_2O)	r.t.	1	Complex RM
11	H_2O	Et_2NH (1:2 v/v to H_2O)	r.t.	1	Complex RM
12	MeOH	Et_3N (3 eq.)	90, µW	0.5	Complex RM
13	Toluene	Et_3N (3 eq.)	110, µW	0.17	Complex RM
14	THF	Et_3N (3 eq.)	65, µW	0.5	15% conversion to **376**
15	THF	Et_3N (1:3 v/v to THF)	90, µW	1	85% isolated yield of **376**



383 384

Figure 20. Proposed structures of side products which were observed during the ring closure reaction screening

The reaction of 1-chloropinacolone with malononitrile and the subsequent ring closure reaction could be merged into a one-pot procedure (Scheme 91). Reaction of 1-chloropinacolone with malononitrile in a mixture of triethylamine and THF at 90 °C under microwave irradiation for one hour resulted in 49% yield of the desired aminofuran **376**. However, the yield of this one-pot reaction is lower than the combined yields of the separate reactions.

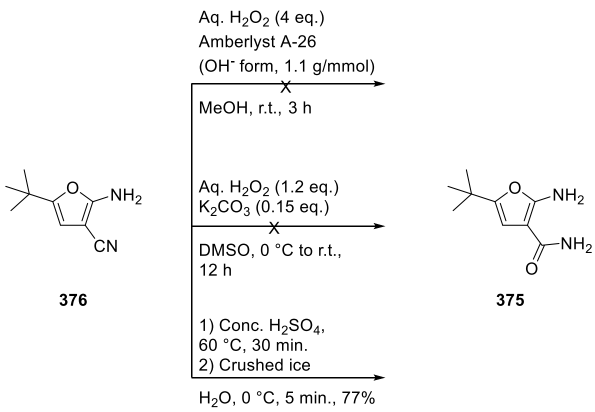


381 376

Scheme 91. One-pot procedure for the synthesis of 2-amino-5-(tert-butyl)furan-3-carbonitrile

3.2 Synthesis of 2-amino-5-(*tert*-butyl)furan-3-carboxamide

The last step in the synthetic pathway to obtain the requested 2-amino-5-(*tert*-butyl)furan-3-carboxamide **375** consisted of the transformation of the nitrile group to an amide functionality. First, a mild, selective and efficient method reported by Lakouraj and coworkers was investigated.[187] The nitrile **376** was reacted with Amberlyst-supported hydroperoxide in methanol, but no conversion of starting material was observed after three hours at room temperature (Scheme 92). The reaction mixture was then heated at 65 °C for half an hour, but this resulted only in the formation of side products and the desired amide **375** was not detected. Katritzky and coworkers reported on the efficient conversion of nitriles to amides using aqueous hydrogen peroxide and potassium carbonate in DMSO at room temperature.[188] When these reaction conditions were applied to nitrile **376**, no conversion was observed after half an hour. Addition of extra aqueous hydrogen peroxide and up to twelve hours of prolonged stirring still did not result in any conversion. After heating of the reaction mixture at 50 °C for three hours, side products were formed and the desired amide **375** was not detected in the reaction mixture. Finally, a classic acid catalyzed hydrolysis was used to convert the nitrile into the amide.[189] Compound **376** was added to concentrated sulfuric acid, heated at 60 °C for half an hour to protonate the nitrile group, and then cooled down to 0 °C. Subsequently, crushed ice was added carefully to affect the hydrolysis. Following this procedure, the amide **375** was obtained in 77% yield. This product appeared to be quite unstable, and during the development of the reaction work-up and purification protocol, the product was lost many times. This is in agreement with literature, which reports that 2-aminofurans are much less stable in the absence of an electron withdrawing group.[190]



*Scheme 92. Conversion of nitrile **376** to amide **375***

3.3 Conclusions

In conclusion, an efficient and straightforward synthetic route towards 2-amino-(5-*tert*-butyl)furan-3-carbonitrile was developed. Starting from commercially available 1-chloropinacolone, a substitution with malononitrile afforded the dinitrile **382** in 86% yield. Ring closure of this product under microwave irradiation gave the 2-aminofuran **376** in 85% yield. Acid catalyzed hydrolysis of the nitrile group to an amide functionality delivered the end product **375** in 77% yield (56% yield over three steps).

4 Drug-likeness of the synthesized compounds

As mentioned in the introduction, the synthesized compounds should preferentially be in agreement with the Lipinski rules for drug-likeness. A selection of relevant compounds was assessed on drug-like properties as defined by the Lipinski Rule of Three (Ro3) and Rule of Five (Ro5) and the results are summarized in Table 17. As can be seen, 25 out of 71 compounds were not in agreement with the Rule of Three. The most frequent property that was not in agreement with the Ro3 was the number of hydrogen bond acceptors (15 out of 25 compounds), followed by the molecular weight (13 out of 25 compounds), the partition coefficient (4 out of 25 compounds) and the number of hydrogen bond donors (3 out of 25 compounds). All of the compounds were in agreement with the Ro5.

Table 17. Assessment of the drug-like properties of the synthesized compounds according to the Lipinski rules. 'Y' = in agreement, 'N' = not in agreement.

Compound	Ro3	Ro5	Compound	Ro3	Ro5
6	Y	Y	**298**	Y	Y
8	Y	Y	**300**	N	Y
284	Y	Y	**299**	Y	Y
36a	Y	Y	**301**	Y	Y
36b	Y	Y	**302**	Y	Y
36c	Y	Y	**305**	N	Y
36d	N	Y	**306**	Y	Y
38a	Y	Y	**314**	N	Y
38b	Y	Y	**316**	N	Y
38c	Y	Y	**317**	Y	Y
38d	Y	Y	**319**	N	Y
38e	Y	Y	**321**	N	Y
38f	Y	Y	**322**	N	Y
38g	Y	Y	**323**	Y	Y
38h	Y	Y	**325**	N	Y
38i	N	Y	**327**	N	Y
38j	N	Y	**328**	N	Y
38k	N	Y	**341**	N	Y
38l	N	Y	**342**	N	Y
38m	Y	Y	**351**	Y	Y
38n	N	Y	**357**	Y	Y
38o	Y	Y	**350a**	Y	Y
285a	Y	Y	**350b**	Y	Y
285b	Y	Y	**350c**	Y	Y
285c	Y	Y	**350d**	Y	Y
285d	Y	Y	**350e**	Y	Y
285e	N	Y	**350f**	Y	Y

285f	Y	Y	**350g**	Y	Y
285g	Y	Y	**337a**	Y	Y
285h	N	Y	**337b**	Y	Y
285i	Y	Y	**337c**	Y	Y
285j	N	Y	**337d**	Y	Y
285k	N	Y	**369**	Y	Y
285l	N	Y	**370**	Y	Y
286	N	Y	**373**	N	Y
288	Y	Y			

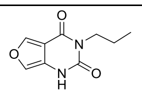
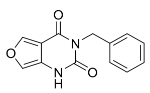
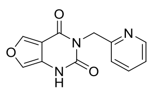
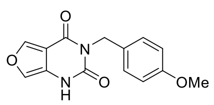
5 Biological evaluation

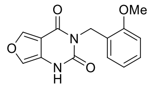
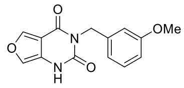
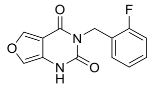
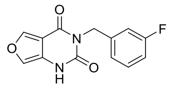
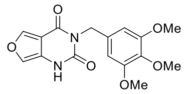
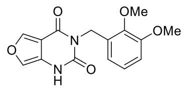
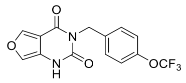
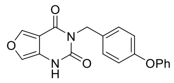
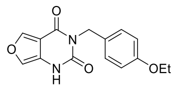
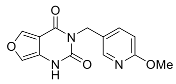
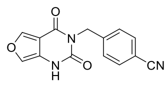
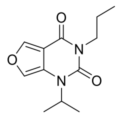
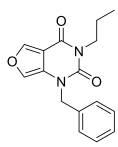
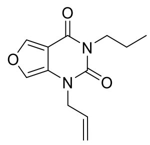
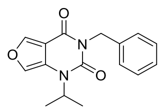
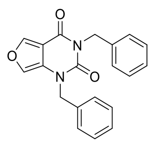
The synthesized compounds were evaluated for biological activity in collaboration with research institutions and companies. There was no clear rationale for the performed biological screenings; whenever there was a call for compounds by a research institution or company, a representative selection of available compounds was submitted for biotesting.

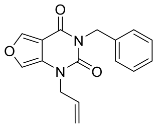
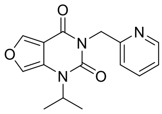
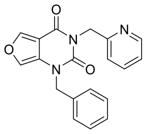
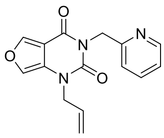
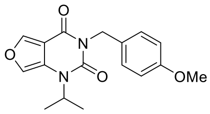
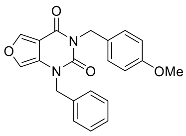
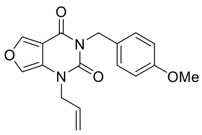
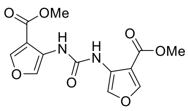
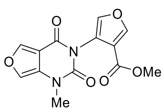
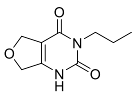
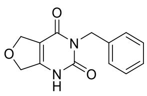
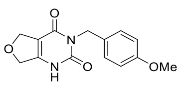
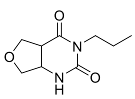
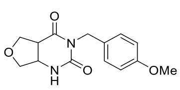
5.1 Inhibition of NPP1

NPP1, or ectonucleotide pyrophosphatase/phosphodiesterase-1, is a membrane glycoprotein that hydrolyzes pyrophosphate and phosphodiester bonds of nucleotides such as ATP. It has a Zn(II)-binding extracellular catalytic site. Hydrolysis of ATP mainly results in the release of AMP and pyrophosphate and also ADP and inorganic phosphate.[191–193] Animal studies suggest that a disrupted NPP1 function is associated with significant changes in skeletal and soft tissue mineralization, the calcium/phosphate balance and glucose homeostasis.[194] In calcific aortic valve disease (CAVD), the most frequent heart valve disorder, an abnormally high level of NPP1 is found,[191,195] and elevated pyrophosphate levels are observed in calcium pyrophosphate deposition disease (CPPD), which is a joint pathology. Both health disorders are lacking disease-modifying therapies. Therefore, new drugs that slow down or stop CAVD/CPPD progression are needed, and selective NPP1 inhibitors could be suitable candidates to treat these diseases.[196–199] The screening of NPP1 inhibition was performed by the research group of Prof. Müller (PharmaCenter Bonn, Pharmaceutical Institute, Pharmaceutical Chemistry I, University of Bonn, An der Immenburg 4, D-53121 Bonn, Germany). The inhibition was determined by assessing the phosphodiesterase activity of NPP1, using *p*-nitrophenyl 5'-thymidine monophosphate (*p*-Nph-5'-TMP) as an artificial substrate. Only compounds which display more than 70% inhibition in this first screening are allowed to proceed to the next screening, which uses the natural substrate ATP. Thirty-four compounds were screened, but none of them were active (Table 18).

Table 18. First screening of NPP1 inhibition. Assay conditions: 400 µM p-nitro-5'-TMP, 10 µM compound concentration, 20 ng of human recombinant NPP1, reaction buffer (1 mM $CaCl_2$, 200 µM $ZnCl_2$, 50 mM Tris, pH 9.0), spectrophotometric detection at 400 nm and n = 1

Compound	Structure	Inhibition of NPP1 (*p*-Nph-5'-TMP)[a]
38a		NA
38b		NA
38c		NA
38d		NA

38e		NA
38f		NA
38g		NA
38h		NA
38i		NA
38j		NA
38k		NA
38l		NA
38m		NA
38n		NA
38o		NA
285a		NA
285b		NA
285c		NA
285d		NA
285e		NA

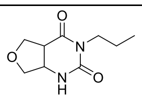
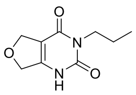
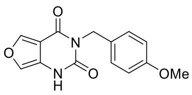
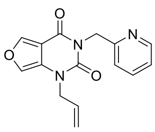
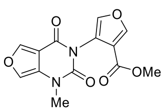
285f		NA
285g		NA
285h		NA
285i		NA
285j		NA
285k		NA
285l		NA
286		NA
288		NA
298		NA
302		NA
300		NA
299		NA
301		NA

[a]: NA = no activity

5.2 Antibacterial activity

Five compounds were submitted to an antibacterial screening campaign of the Laboratory for Microbiology at the University of Ghent (K.L. Ledeganckstraat 35, 9000 Gent, Belgium) in collaboration with the Belgian Co-ordinated Collections of Micro-organisms (BCCM). The growth inhibition was tested on a panel of four internationally used test- and control strains based on clinical relevance. The four strains were the Gram-negative *Escherichia coli* LMG 8063 and *Klebsiella pneumoniae* LMG 2095 and the Gram-positive *Staphylococcus aureus* LMG 8064 and *Bacillus subtilis* LMG 13579. The initial screening used a single ‘high’ concentration (C, Table 19) against the test panel and was based on visual assessment of bacterial growth compared to negative and positive controls. None of the compounds was active against the Gram-negative strains (Table 19). Compounds **299**, **298** and **285i** showed a very slight growth inhibition of both of the Gram-positive strains.

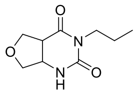
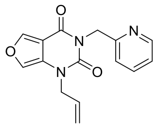
Table 19. First screening of antibacterial activity

Compound	Structure	C (µg/mL)	Growth inhibition[a]			
			EC	*KP*	*SA*	*BS*
			LMG 8063	LMG 2095	LMG 8064	LMG 13579
299		508	NA	NA	Very slight	Very slight
298		194	NA	NA	Very slight	Very slight
38d		490	NA	NA	NA	NA
285i		520	NA	NA	Very slight	Very slight
288		502	NA	NA	NA	NA

[a]: NA = no activity

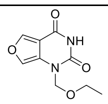
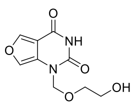
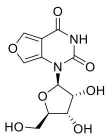
Compounds **299** and **285i** were selected to proceed to a follow-up assay to determine their MIC values against four clinically relevant Gram-positive strains: *Enterococcus faecium* LMG 11397, *Streptococcus pneumoniae* LMG 16738, *Staphylococcus aureus* LMG 8064 and *Bacillus subtilis* LMG 13579. Both compounds exhibited MIC values of >1000 µg/mL for all four bacterial strains (Table 20).

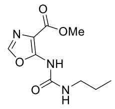
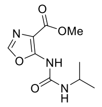
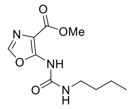
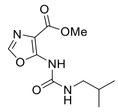
Table 20. Determination of MIC values for selected compounds against four Gram-positive strains

Compound	Structure	MIC (µg/mL)			
		EF	*SP*	*SA*	*BS*
		LMG 11397	LMG 16738	LMG 8064	LMG 13579
Positive control gentamicin		5	5	25	10
299		>1000	>1000	>1000	>1000
285i		>1000	>1000	>1000	>1000

Seven compounds were submitted to a screening campaign of the Laboratory of Pharmaceutical Microbiology (Prof. Coenye, Ghent University). The purpose of the campaign was the identification of new agents with activity against bacterial biofilms. Bacteria usually don't exist as individual organisms in a 'planktonic state', but can form monolayers, multilayer colonies and finally a mature biofilm. The extracellular matrix or 'slime' which forms the basis for the biofilm consists of extracellular polysaccharides, cell debris, nucleic acids and structural proteins, also called extracellular polymeric substances (EPS). Bacterial biofilms are much more resistant to antibiotics, components of both the innate and adaptive immune response and phagocytosis. Hence, they are very hard to treat with antibiotics.[200] Before the compounds were allowed to proceed to the antibacterial biofilm assay, a first screening assessed their antibacterial activity against the Gram-negative bacterial strains *Escherichia coli* LMG 8063, *Klebsiella pneumoniae* LMG 2095 and *Pseudomonas aeruginosa* PAO1, and the Gram-positive strains *Staphylococcus aureus* ATCC 6538 and *Staphylococcus aureus* Mu50, which is a multi-resistant strain. The screening assay was carried out in 96-well plates in a concentration range of 2500 to 1.22 µg/mL of test compound. None of the compounds exhibited antibacterial activity at the tested concentrations (Table 21).

Table 21. Screening for antibacterial activity

		Growth inhibition[a]				
Compound	Structure	*EC*	*KP*	*PA*	*SA*	*SA*
		LMG 8063	LMG 2095	PAO1	ATCC 6538	Mu50
317		NA	NA	NA	NA	NA
316		NA	NA	NA	NA	NA
314		NA	NA	NA	NA	NA

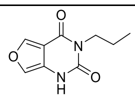
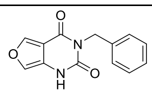
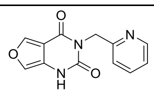
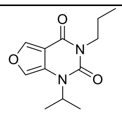
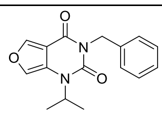
350a		NA	NA	NA	NA	NA
350c		NA	NA	NA	NA	NA
350b		NA	NA	NA	NA	NA
350e		NA	NA	NA	NA	NA

[a]: NA = no activity

5.3 Antiviral activity

The feline parvovirus (FPV) is a single-stranded DNA virus which causes panleukopenia in both domestic and wild feline species. It is also known as ‘cat plague’ and is a highly contagious and often lethal disease.[201] The activity against FPV was tested on CrFK infected cell cultures by Okapi Sciences, Leuven, Belgium. Five compounds were screened at concentrations of 50, 10, 2 and 0.4 µM and none of them were active against FPV (Table 22). The screening campaign was terminated by Okapi Sciences and no more compounds were tested.

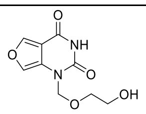
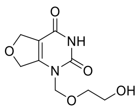
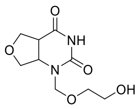
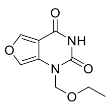
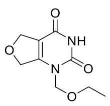
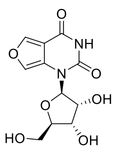
Table 22. Screening for activity against Feline Parvovirus

Compound	Structure	Activity against FPV
38a		NA
38b		NA
38c		NA
285a		NA
285d		NA

[a]: NA = no activity

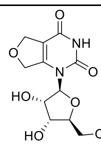
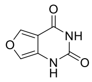
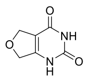
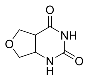
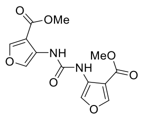
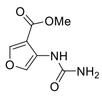
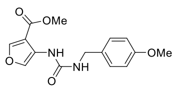
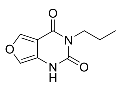
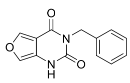
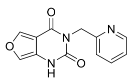
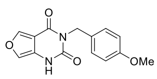
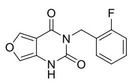
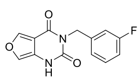
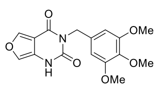
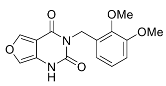
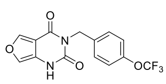
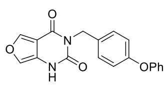
A collection of 44 compounds was screened for antiviral activity against a broad panel of viruses in different cell cultures (Tables 23-27). This screening was performed by prof. Naesens of the Rega Institute for Medical Research (KU Leuven). The activity against vesicular stomatitis virus, coxsackie virus B4 and RSV was determined in either HeLa cell cultures (Table 23) or Hep-2 cell cultures (Table 24). In the HeLa cell culture assay, compounds **321** and **327** exhibited a slight activity against coxsackie virus B4, with EC_{50} values of **97** and **67** µM, respectively. Both structures are characterized by a (2-hydroxyethoxy)methyl substituent on *N*-1. Compound **316**, also equipped with this substituent, showed no activity. Compound **38k** was slightly cytotoxic in Hep-2 cell cultures with a CC50 of 89 µM. The activity against HIV-1 and HIV-2 was determined in MT4 cell cultures (Table 25). Only compound **305** was slightly active against HIV-1 strain IIIB with an EC_{50} value of 104 µM. Compounds **38k** and **38l** were slightly cytotoxic with CC_{50} values of 64 and 69 µM, respectively. The activity against herpes simplex virus-1 strain KOS, herpes simplex virus-2 strain G, thymidine kinase negative and acyclovir-resistant herpes simplex virus-1 strain KOS, vaccinia virus, adenovirus-2 and human coronavirus strain 229E was determined in HEL cell cultures (Table 26). None of the compounds showed antiviral activity in this assay. The activity against reovirus-1, sindbis virus, coxsackie virus B4, punta toro virus and zika virus was determined in Vero cell cultures (Table 27). In this assay, none of the compounds showed antiviral activity. Compounds **321** and **327**, which exhibited slight activity against coxsackie virus B4 in HeLa cell cultures, did not possess activity against this virus in Vero cell cultures.

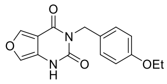
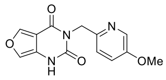
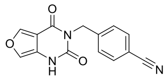
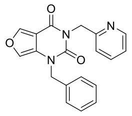
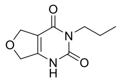
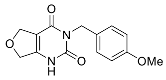
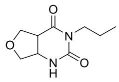
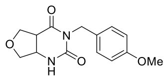
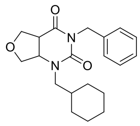
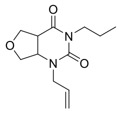
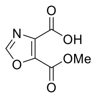
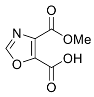
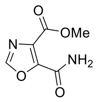
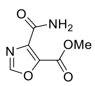
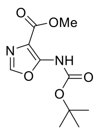
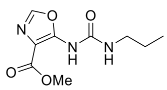
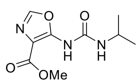
Table 23. Cytotoxicity and antiviral activity in HeLa cell cultures

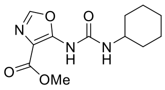
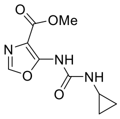
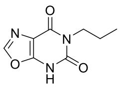
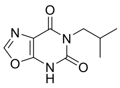
Compound	Structure	CC_{50}[a] (µM)	Antiviral EC_{50}[b] (µM)		
			Vesicular stomatitis virus	Coxsackie virus B4	Respiratory syncytial virus
316		> 100	> 100	> 100	> 100
321		> 100	> 100	97	> 100
327		> 100	> 100	67	> 100
317		> 100	> 100	> 100	> 100
322		> 100	> 100	> 100	> 100
314		> 100	> 100	> 100	> 100
DS-10.000		> 100[c]	0.3[c]	> 100[c]	0.2[c]
Ribavirin		> 250	87	126	5

[a]:50% Cytotoxic concentration, as determined by measuring the cell viability with the colorimetric formazan-based MTS assay. [b]:50% Effective concentration, or concentration producing 50% inhibition of virus-induced cytopathic effect, as determined by measuring the cell viability with the colorimetric formazan-based MTS assay. [c]: in µg/mL.

Table 24. Cytotoxicity and antiviral activity in Hep-2 cell cultures

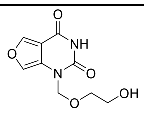
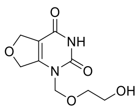
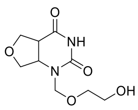
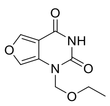
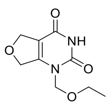
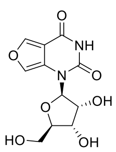
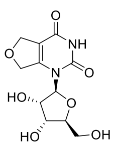
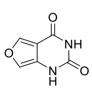
Compound	Structure	CC_{50}[a] (µM)	Antiviral EC_{50}[b] (µM)		
			Vesicular stomatitis virus	Coxsackie virus B4	Respiratory syncytial virus
319		>100	>100	>100	>100
6		>100	>100	>100	>100
8		>100	>100	>100	>100
323		>100	>100	>100	>100
286		>100	>100	>100	>100
284		>100	>100	>100	>100
36d		>100	>100	>100	>100
38a		>100	>100	>100	>100
38b		>100	>100	>100	>100
38c		>100	>100	>100	>100
38d		>100	>100	>100	>100
38g		>100	>100	>100	>100
38h		>100	>100	>100	>100
38i		>100	>100	>100	>100
38j		>100	>100	>100	>100
38k		89	>100	>100	>100
38l		>100	>100	>100	>100

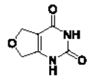
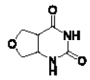
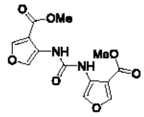
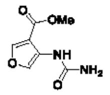
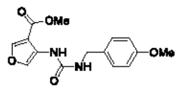
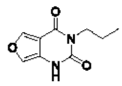
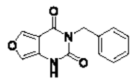
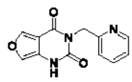
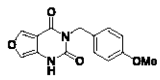
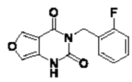
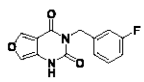
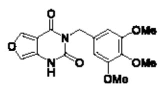
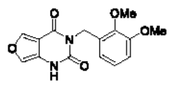
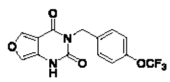
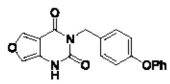
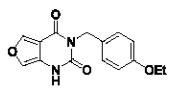
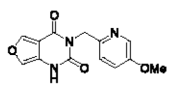
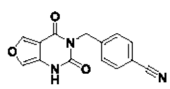
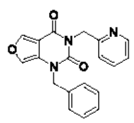
38m		>100	>100	>100	>100
38n		>100	>100	>100	>100
38o		>100	>100	>100	>100
285h		>100	>100	>100	>100
298		>100	>100	>100	>100
300		>100	>100	>100	>100
299		>100	>100	>100	>100
301		>100	>100	>100	>100
305		>100	>100	>100	>100
306		>100	>100	>100	>100
341		>100	>100	>100	>100
342		>100	>100	>100	>100
351		>100	>100	>100	>100
357		>100	>100	>100	>100
369		>100	>100	>100	>100
350a		>100	>100	>100	>100
350c		>100	>100	>100	>100

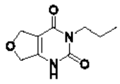
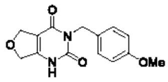
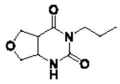
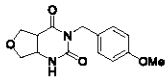
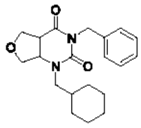
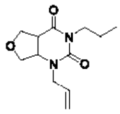
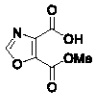
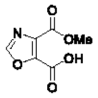
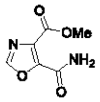
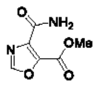
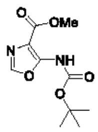
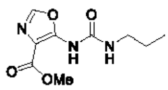
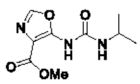
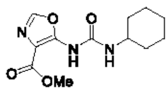
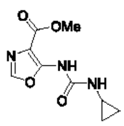
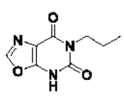
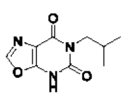
350f		>100	>100	>100	>100
350g		>100	>100	>100	>100
337a		>100	>100	>100	>100
337c		>100	>100	>100	>100
DS-10.000		> 100[c]	0.07[c]	> 100[c]	0.05[c]
Ribavirin		> 250	22	112	4.5

[a]:50% Cytotoxic concentration, as determined by measuring the cell viability with the colorimetric formazan-based MTS assay. [b]:50% Effective concentration, or concentration producing 50% inhibition of virus-induced cytopathic effect, as determined by measuring the cell viability with the colorimetric formazan-based MTS assay. [c]: in µg/mL.

Table 25. Anti-HIV activity and cytotoxicity in MT4 cells

Compound	Structure	CC_{50}[a] (µM)	Antiviral EC_{50}[b] (µM)	
			HIV-1 (strain IIIB)	HIV-2 (strain ROD)
316		> 125	> 125	> 125
321		> 125	> 125	> 125
327		> 125	> 125	> 125
317		> 125	> 125	> 125
322		> 125	> 125	> 125
314		> 125	> 125	> 125
319		> 125	> 125	> 125
6		> 125	> 125	> 125

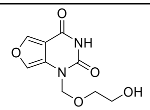
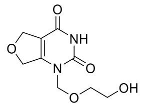
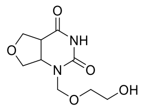
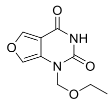
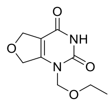
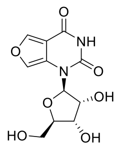
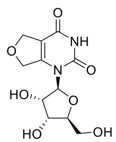
8		> 125	> 125	> 125
323		> 125	> 125	> 125
286		> 125	> 125	> 125
284		> 125	> 125	> 125
36d		> 125	> 125	> 125
38a		> 125	> 125	> 125
38b		> 125	> 125	> 125
38c		> 125	> 125	> 125
38d		> 125	> 125	> 125
38g		> 125	> 125	> 125
38h		> 125	> 125	> 125
38i		> 125	> 125	> 125
38j		> 125	> 125	> 125
38k		64	> 64	> 64
38l		69	> 69	> 69
38m		> 125	> 125	> 125
38n		> 125	> 125	> 125
38o		> 125	> 125	> 125
285h		> 125	> 125	> 125

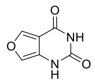
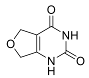
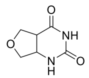
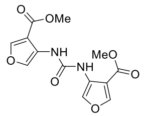
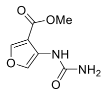
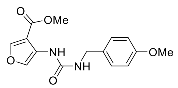
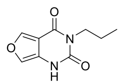
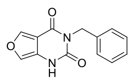
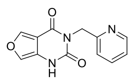
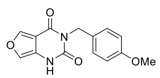
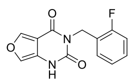
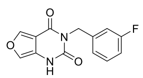
298		> 125	> 125	> 125
300		> 125	> 125	> 125
299		> 125	> 125	> 125
301		> 125	> 125	> 125
305		> 125	104	> 125
306		> 125	> 125	> 125
341		> 125	> 125	> 125
342		> 125	> 125	> 125
351		> 125	> 125	> 125
357		> 125	> 125	> 125
369		> 125	> 125	> 125
350a		> 125	> 125	> 125
350c		> 125	> 125	> 125
350f		> 125	> 125	> 125
350g		> 125	> 125	> 125
337a		> 125	> 125	> 125
337c		> 125	> 125	> 125

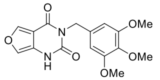
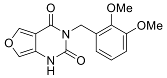
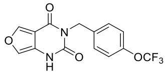
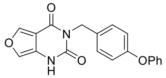
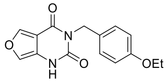
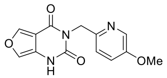
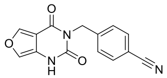
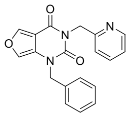
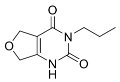
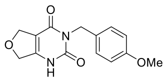
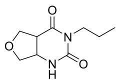
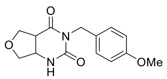
Nevirapine	>4[a]	0.075[a]	>4[a]
Zidovudine	>2[a]	0.0020[a]	0.0022[a]
Lamivudine	>20[a]	0.58[a]	2.3[a]
Didanosine	>50[a]	18[a]	19[a]

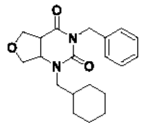
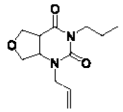
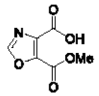
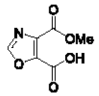
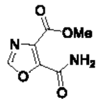
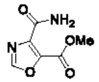
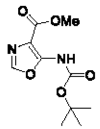
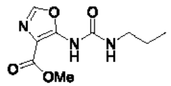
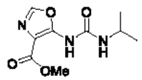
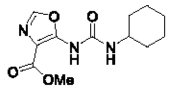
Antiviral activity and cytotoxicity were determined by the colorimetric MTT cell viability assay. The antiviral EC_{50} represents the compound concentration producing 50% inhibition of virus-induced cytopathicity. The CC_{50} represents the compound concentration causing 50% reduction of cell viability. [a]: in µg/mL.

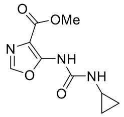
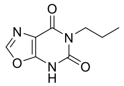
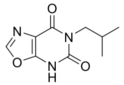
Table 26. Cytotoxicity and antiviral activity in HEL cell cultures

Compound	Structure	CC_{50}[a] (µM)	Antiviral EC_{50}[b] (µM)					
			Herpes simplex virus-1 (KOS)	Herpes simplex virus-2 (G)	Herpes simplex virus-1 TK$^-$ KOS ACV^r	Vaccinia virus	Adenovirus-2	Human coronavirus (229E)
316		> 100	> 100	> 100	> 100	> 100	> 100	> 100
321		> 100	> 100	> 100	> 100	> 100	> 100	> 100
327		> 100	> 100	> 100	> 100	> 100	> 100	> 100
317		> 100	> 100	> 100	> 100	> 100	> 100	> 100
322		> 100	> 100	> 100	> 100	> 100	> 100	> 100
314		> 100	> 100	> 100	> 100	> 100	> 100	> 100
319		> 100	> 100	> 100	> 100	> 100	> 100	> 100

6		> 100	> 100	> 100	> 100	> 100	> 100	> 100
8		> 100	> 100	> 100	> 100	> 100	> 100	> 100
323		> 100	> 100	> 100	> 100	> 100	> 100	> 100
286		> 100	> 100	> 100	> 100	> 100	> 100	> 100
284		> 100	> 100	> 100	> 100	> 100	> 100	> 100
36d		> 100	> 100	> 100	> 100	> 100	> 100	> 100
38a		> 100	> 100	> 100	> 100	> 100	> 100	> 100
38b		> 100	> 100	> 100	> 100	> 100	> 100	> 100
38c		> 100	> 100	> 100	> 100	> 100	> 100	> 100
38d		> 100	> 100	> 100	> 100	> 100	> 100	> 100
38g		> 100	> 100	> 100	> 100	> 100	> 100	> 100
38h		> 100	> 100	> 100	> 100	> 100	> 100	> 100

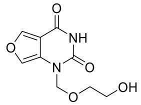
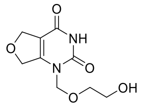
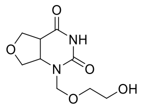
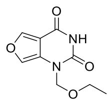
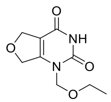
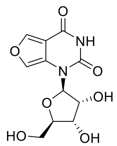
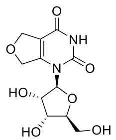
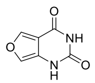
38i		> 100	> 100	> 100	> 100	> 100	> 100	> 100
38j		> 100	> 100	> 100	> 100	> 100	> 100	> 100
38k		> 100	> 100	> 100	> 100	> 100	> 100	> 100
38l		> 100	> 100	> 100	> 100	> 100	> 100	> 100
38m		> 100	> 100	> 100	> 100	> 100	> 100	> 100
38n		> 100	> 100	> 100	> 100	> 100	> 100	> 100
38o		> 100	> 100	> 100	> 100	> 100	> 100	> 100
285h		> 100	> 100	> 100	> 100	> 100	> 100	> 100
298		> 100	> 100	> 100	> 100	> 100	> 100	> 100
300		> 100	> 100	> 100	> 100	> 100	> 100	> 100
299		> 100	> 100	> 100	> 100	> 100	> 100	> 100
301		> 100	> 100	> 100	> 100	> 100	> 100	> 100

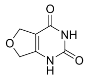
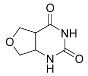
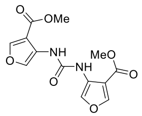
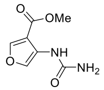
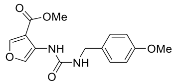
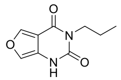
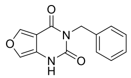
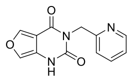
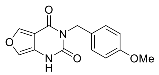
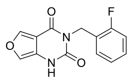
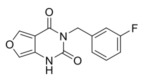
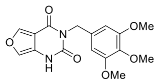
305		> 100	> 100	> 100	> 100	> 100	> 100	> 100
306		> 100	> 100	> 100	> 100	> 100	> 100	> 100
341		> 100	> 100	> 100	> 100	> 100	> 100	> 100
342		> 100	> 100	> 100	> 100	> 100	> 100	> 100
351		> 100	> 100	> 100	> 100	> 100	> 100	> 100
357		> 100	> 100	> 100	> 100	> 100	> 100	> 100
369		> 100	> 100	> 100	> 100	> 100	> 100	> 100
350a		> 100	> 100	> 100	> 100	> 100	> 100	> 100
350c		ND	> 100	> 100	> 100	> 100	> 100	> 100
350f		> 100	> 100	> 100	> 100	> 100	> 100	> 100

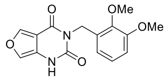
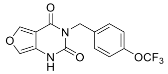
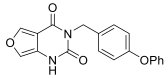
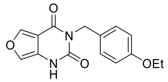
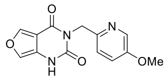
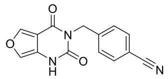
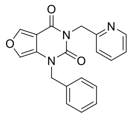
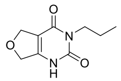
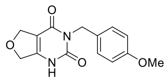
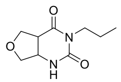
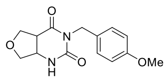
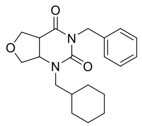
350g		> 100	> 100	> 100	> 100	> 100	> 100	> 100
337a		> 100	> 100	> 100	> 100	> 100	> 100	> 100
337c		> 100	> 100	> 100	> 100	> 100	> 100	> 100
Brivudin		> 250	0.1	96	>250	13		
Cidofovir		> 250	32	7.5	21	8.7	11	
Acyclovir		> 250	2.9	0.1	>250	>250		
Ganciclovir		> 100	0.7	0.7	4.8	>100		
Zalcitabine		>250					17	
Alovudine		>250					9.3	
UDA		> 100[c]						3.9[c]

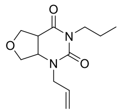
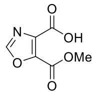
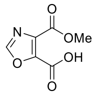
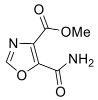
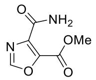
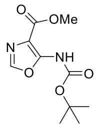
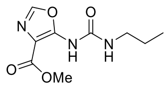
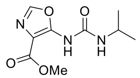
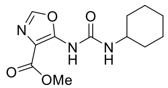
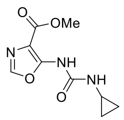
[a]:50% Cytotoxic concentration, as determined by measuring the cell viability with the colorimetric formazan-based MTS assay. [b]:50% Effective concentration, or concentration producing 50% inhibition of virus-induced cytopathic effect, as determined by measuring the cell viability with the colorimetric formazan-based MTS assay. [c]: in µg/mL. ND = not determined

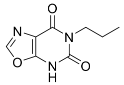
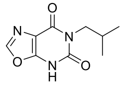
Table 27. Cytotoxicity and antiviral activity in Vero cell cultures

Compound	Structure	CC_{50}[a] (µM)	Antiviral EC_{50}[b] (µM)				
			Reovirus-1	Sindbis virus	Coxsackie virus B4	Punta Toro virus	Zika virus
316		> 100	> 100	> 100	> 100	> 100	> 100
321		> 100	> 100	> 100	> 100	> 100	> 100
327		> 100	> 100	> 100	> 100	> 100	> 100
317		> 100	> 100	> 100	> 100	> 100	> 100
322		> 100	> 100	> 100	> 100	> 100	> 100
314		> 100	> 100	> 100	> 100	> 100	> 100
319		> 100	> 100	> 100	> 100	> 100	> 100
6		> 100	> 100	> 100	> 100	> 100	> 100

8		> 100	> 100	> 100	> 100	> 100	> 100
323		> 100	> 100	> 100	> 100	> 100	> 100
286		> 100	> 100	> 100	> 100	> 100	> 100
284		> 100	> 100	> 100	> 100	> 100	> 100
36d		> 100	> 100	> 100	> 100	> 100	> 100
38a		> 100	> 100	> 100	> 100	> 100	> 100
38b		> 100	> 100	> 100	> 100	> 100	> 100
38c		> 100	> 100	> 100	> 100	> 100	> 100
38d		> 100	> 100	> 100	> 100	> 100	> 100
38g		> 100	> 100	> 100	> 100	> 100	> 100
38h		> 100	> 100	> 100	> 100	> 100	> 100
38i		> 100	> 100	> 100	> 100	> 100	> 100

38j		> 100	> 100	> 100	> 100	> 100	> 100
38k		> 100	> 100	> 100	> 100	> 100	> 100
38l		> 100	> 100	> 100	> 100	> 100	> 100
38m		> 100	> 100	> 100	> 100	> 100	> 100
38n		> 100	> 100	> 100	> 100	> 100	> 100
38o		> 100	> 100	> 100	> 100	> 100	> 100
285h		> 100	> 100	> 100	> 100	> 100	> 100
298		> 100	> 100	> 100	> 100	> 100	> 100
300		> 100	> 100	> 100	> 100	> 100	> 100
299		> 100	> 100	> 100	> 100	> 100	> 100
301		> 100	> 100	> 100	> 100	> 100	> 100
305		> 100	> 100	> 100	> 100	> 100	> 100

306		> 100	> 100	> 100	> 100	> 100	> 100
341		> 100	> 100	> 100	> 100	> 100	> 100
342		> 100	> 100	> 100	> 100	> 100	> 100
351		> 100	> 100	> 100	> 100	> 100	> 100
357		> 100	> 100	> 100	> 100	> 100	> 100
369		> 100	> 100	> 100	> 100	> 100	> 100
350a		> 100	> 100	> 100	> 100	> 100	> 100
350c		> 100	> 100	> 100	> 100	> 100	> 100
350f		> 100	> 100	> 100	> 100	> 100	> 100
350g		> 100	> 100	> 100	> 100	> 100	> 100

337a		> 100	> 100	> 100	> 100	> 100	> 100
337c		> 100	> 100	> 100	> 100	> 100	> 100
DS-10,000		>100[c]	1.2[c]	14.0[c]	26.2[c]	5.4[c]	24.1[c]
Mycophenolic acid		>100	>100	21.5	>100	10.6	10.1

[a]:50% Cytotoxic concentration, as determined by measuring the cell viability with the colorimetric formazan-based MTS assay. [b]:50% Effective concentration, or concentration producing 50% inhibition of virus-induced cytopathic effect, as determined by measuring the cell viability with the colorimetric formazan-based MTS assay. [c]: in µg/mL.

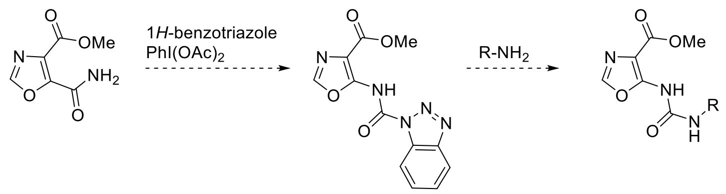
5.4 Conclusions

A variety of compounds which were synthesized during this PhD research were screened for antibacterial and antiviral activity, and inhibition of NPP1. No significant activity was found in these screenings. A slight antibacterial activity was observed for three compounds, but determination of the MIC values for two of these compounds resulted in values higher than 1000 µg/mL. A slight antiviral activity was observed for two acyclic nucleoside analogues against coxsackie virus B4 in HeLa cell cultures, but these compounds did not exhibit this activity in Vero cell cultures. One compound was slightly active against HIV-1 strain IIIB. The activity of these hits was not good enough to start a hit optimization campaign. Thus, it can be concluded that the biological screening of the compound libraries did not return any significant hits.

Perspectives

The proposed furo[3,4-*d*]pyrimidines were not obtained because the transformation of the furo[3,4-*d*]pyrimidine-2,4-dione precursors by treatment with phosphorus oxychloride was not successful. Other reagents might be able to accomplish the transformation to furo[3,4-d]pyrimidines, and a more thorough screening of reagents could be performed. However, it is possible that this approach is chemically intractable, and that the furo[3,4-*d*]pyrimidines should be constructed *via* ring annulation of appropriate pyrimidine precursors instead.

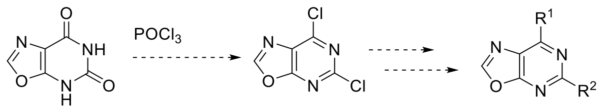
A handful of oxazolo[5,4-*d*]pyrimidine-5,7-diones was synthesized, but the scope of the Hofmann reaction was limited to amines that were not very sensitive to oxidation by the hypervalent iodine reagent. This problem could be tackled by introducing an extra step in the reaction pathway and applying the Hofmann rearrangement reaction for the synthesis of an appropriate precursor for the ureids. The (oxidation-sensitive) amines could then be allowed to react with this precursor under non-oxidative conditions to afford the ureids. A possible precursor could be the benzotriazole carboxamide **385** (Scheme 93). The successful *in situ* trapping of isocyanates (obtained via Curtius rearrangement) with 1*H*-benzotriazole to form this type of benzotriazole carboxamides, and the subsequent reaction with amines to afford ureid compounds, has been described already.[202] This approach should allow the use of a much broader range of amines, so that a diverse compound library of oxazolo[5,4-*d*]pyrimidine-5,7-diones can be designed and synthesized.



351 **385** **350**

*Scheme 93. Transformation of carboxamide **351** to ureid **350** via a benzotriazole ureid precursor*

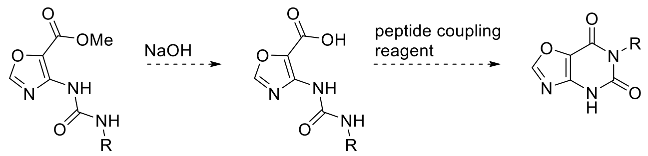
Patent literature strongly suggests that the transformation of oxazolo[5,4-*d*]pyrimidine-5,7-dione **337e**. to the 5,7-dichlorooxazolo[5,4-*d*]pyrimidine scaffold **388** by treatment with phosphorus oxychloride is synthetically feasible, as well as further modification towards the functionalized oxazolo[5,4-*d*]pyrimidines **389** (Scheme 94).[56]



337e **388** **389**

*Scheme 94. Proposed synthetic pathway towards the C-5/C-7 functionalized oxazolo[5,4-d]pyrimidines **389***

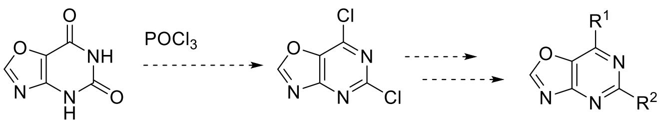
The oxazolo[4,5-*d*]pyrimidine-5,7-dione **333a** was obtained only in a very low yield. The ring closure protocol for the ureids **370** to oxazolo[4,5-*d*]pyrimidinones **333** could be optimized to improve the yield, or another approach could be followed. A possible new approach could be the selective hydrolysis of the ester bond of ureid **370** to obtain the carboxylic acid **390** (Scheme 95). Ring closure could then be accomplished under peptide coupling conditions, for example with PyBOP reagent, as reported by Drewe and coworkers.[203]



370 **390** **333**

*Scheme 95. Proposed synthetic strategy to ring-close ureids **370** towards the oxazolo[4,5-d]pyrimidine-5,7-diones **333***

Patent literature strongly suggests that oxazolo[4,5-*d*]pyrimidine-5,7-dione **333a** could be converted to the 5,7-dichlorooxazolo[4,5-*d*]pyrimidine scaffold **393** by treatment with phosphorus oxychloride, and subsequent modification would then afford the functionalized oxazolo[4,5-*d*]pyrimidines **394** (Scheme 96).[56]



333a **393** **394**

*Scheme 96. Proposed synthetic pathway towards the oxazolo[4,5-d]pyrimidines **394***

None of the screened compounds possessed a significant biological activity which would justify further medicinal optimization. However, the library of new compounds can be used for further biological screening in the future.

Experimental part

1 Materials and methods

All of the reagents and solvents were commercially available and used as such, unless mentioned otherwise.

1.1 Relevant safety data sheet information

1.1.1 Methanol

Hazard statements:

H225 Highly flammable liquid and vapour.

H301 + H311 + H331 Toxic if swallowed, in contact with skin or if inhaled.

H370 Causes damage to organs.

Precautionary statements:

P210 Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.

P280 Wear protective gloves/ protective clothing.

P302 + P352 + P312 IF ON SKIN: Wash with plenty of water. Call a POISON CENTER/doctor if you feel unwell.

P304 + P340 + P311 IF INHALED: Remove person to fresh air and keep comfortable for breathing. Call a POISON CENTER/doctor.

P370 + P378 In case of fire: Use dry powder or dry sand to extinguish.

P403 + P235 Store in a well-ventilated place. Keep cool.

1.1.2 Sodium hydroxide

Hazard statements:

H290 May be corrosive to metals.

H314 Causes severe skin burns and eye damage.

Precautionary statements:

P260 Do not breathe dust/ fume/ gas/ mist/ vapours/ spray.

P280 Wear protective gloves/ protective clothing/ eye protection/ face protection.

P303 + P361 + P353 IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water/shower.

P304 + P340 + P310 IF INHALED: Remove person to fresh air and keep comfortable for breathing. Immediately call a POISON CENTER/doctor.

P305 + P351 + P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

1.1.3 Diphenyl phosphoryl azide

Hazard statements:

H301 + H311 + H331 Toxic if swallowed, in contact with skin or if inhaled.

H315 Causes skin irritation.

H319 Causes serious eye irritation.

H335 May cause respiratory irritation.

Precautionary statements:

P261 Avoid breathing vapours.

P280 Wear protective gloves/ protective clothing.

P301 + P310 IF SWALLOWED: Immediately call a POISON CENTER/doctor.

P305 + P351 + P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

P311 Call a POISON CENTER /doctor.

1.1.4 Triethylamine

Hazard statements:

H225 Highly flammable liquid and vapour.

H302 Harmful if swallowed.

H311 + H331 Toxic in contact with skin or if inhaled.

H314 Causes severe skin burns and eye damage.

H335 May cause respiratory irritation.

Precautionary statements:

P210 Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.

P261 Avoid breathing dust/ fume/ gas/ mist/ vapours/ spray.

P280 Wear protective gloves/ protective clothing/ eye protection/ face protection.

P303 + P361 + P353 IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water/shower.

P305 + P351 + P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

P370 + P378 In case of fire: Use dry sand, dry chemical or alcohol-resistant foam to extinguish.

1.1.5 Sodium methoxide

Hazard statements:

H251 Self-heating: may catch fire.

H302 Harmful if swallowed.

H314 Causes severe skin burns and eye damage.

Precautionary statements:

P235 + P410 Keep cool. Protect from sunlight.

P280Wear protective gloves/ protective clothing/ eye protection/ face protection.

P305 + P351 + P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

P310 Immediately call a POISON CENTER/doctor.

Supplemental Hazard information (EU):

EUH014 Reacts violently with water.

Other hazards:

This substance/mixture contains no components considered to be either persistent, bioaccumulative and toxic (PBT), or very persistent and very bioaccumulative (vPvB) at levels of 0.1% or higher. Reacts violently with water.

1.1.6 Phosphorus(V) oxychloride

Hazard statements:

H300 + H330 Fatal if swallowed or if inhaled

H314 Causes severe skin burns and eye damage.

H372 Causes damage to organs through prolonged or repeated exposure.

Precautionary statements:

P260 Do not breathe dust/ fume/ gas/ mist/ vapours/ spray.

P280 Wear protective gloves/ protective clothing/ eye protection/ face protection.

P301 + P310 + P330 IF SWALLOWED: Immediately call a POISON CENTER/doctor. Rinse mouth.

P303 + P361 + P353 IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water/shower.

P304 + P340 + P310 IF INHALED: Remove person to fresh air and keep comfortable for breathing. Immediately call a POISON CENTER/doctor.

P305 + P351 + P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

P403 + P233 Store in a well-ventilated place. Keep container tightly closed.

Supplemental Hazard information (EU):

EUH014 Reacts violently with water.

EUH029Contact with water liberates toxic gas.

Other hazards:

This substance/mixture contains no components considered to be either persistent, bioaccumulative and toxic (PBT), or very persistent and very bioaccumulative (vPvB) at levels of 0.1% or higher. Reacts violently with water. Contact with water liberates toxic gas. Lachrymator.

1.1.7 Triethylsilane

Hazard statements:

H225 Highly flammable liquid and vapour.

H412 Harmful to aquatic life with long lasting effects.

Precautionary statements:

P210 Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.

P273 Avoid release to the environment

1.1.8 Trifluoroacetic acid

Hazard statements:

H314 Causes severe skin burns and eye damage.

H332 Harmful if inhaled.

H412 Harmful to aquatic life with long lasting effects.

Precautionary statements:

P261 Avoid breathing dust/ fume/ gas/ mist/ vapours/ spray.

P271 Use only outdoors or in a well-ventilated area.

P273 Avoid release to the environment.

P304 + P340 + P312 IF INHALED: Remove person to fresh air and keep comfortable for breathing. Call a POISON CENTER/doctor if you feel unwell.

P501 Dispose of contents/ container to an approved waste disposal plant.

1.1.9 *N*,*N*-Dimethylformamide

Hazard statements:

H226 Flammable liquid and vapour.

H312 + H332 Harmful in contact with skin or if inhaled.

H319 Causes serious eye irritation.

H360D May damage the unborn child.

Precautionary statements:

P201 Obtain special instructions before use.

P280 Wear protective gloves/ protective clothing.

P305 + P351 + P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

P308 + P313 IF exposed or concerned: Get medical advice/ attention.

1.1.10 Trimethylsilyl trifluoromethanesulfonate

Hazard statements:

H226 Flammable liquid and vapour.

H314 Causes severe skin burns and eye damage.

Precautionary statements:

P280 Wear protective gloves/ protective clothing/ eye protection/ face protection.

P305 + P351 + P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

P310 Immediately call a POISON CENTER/doctor.

Supplemental Hazard information (EU):

EUH014 Reacts violently with water.

1.1.11 ***N*****,*****O*****-Bis(trimethylsilyl)acetamide**

Hazard statements:

H226 Flammable liquid and vapour.

H302 Harmful if swallowed.

H314 Causes severe skin burns and eye damage.

Precautionary statements:

P280 Wear protective gloves/ protective clothing/ eye protection/ face protection.

P305 + P351 + P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

P310 Immediately call a POISON CENTER/doctor.

Supplemental Hazard information (EU):

EUH014 Reacts violently with water.

1.1.12 Oxalyl chloride

Hazard statements:

H314 Causes severe skin burns and eye damage.

H331 Toxic if inhaled.

H335 May cause respiratory irritation.

Precautionary statements:

P261 Avoid breathing vapours.

P280 Wear protective gloves/ protective clothing/ eye protection/ face protection.

P305 + P351 + P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

P310 Immediately call a POISON CENTER/doctor.

Supplemental Hazard information (EU):

EUH014 Reacts violently with water.

EUH029 Contact with water liberates toxic gas

1.1.13 Sodium azide

Hazard statements:

H300 + H310 Fatal if swallowed or in contact with skin.

H373 May cause damage to organs (Brain) through prolonged or repeated exposure if swallowed.

H410 Very toxic to aquatic life with long lasting effects.

Precautionary statements:

P273 Avoid release to the environment.

P280 Wear protective gloves/ protective clothing/ eye protection/ face protection.

P301 + P310 + P330 IF SWALLOWED: Immediately call a POISON CENTER/doctor. Rinse mouth.

P302 + P352 + P310 IF ON SKIN: Wash with plenty of water. Immediately call a POISON CENTER/doctor.

P391 Collect spillage.

P501 Dispose of contents/ container to an approved waste disposal plant.

Supplemental Hazard information (EU):

EUH032 Contact with acids liberates very toxic gas.

This substance/mixture contains no components considered to be either persistent, bioaccumulative and toxic (PBT), or very persistent and very bioaccumulative (vPvB) at levels of 0.1% or higher. Contact with acids liberates very toxic gas. Sodium Azide may react with lead and copper plumbing to form highly explosive metal azides. Rapidly absorbed through skin.

1.1.14 Malononitrile

Hazard statements:

H301 + H311 + H331 Toxic if swallowed, in contact with skin or if inhaled.

H410 Very toxic to aquatic life with long lasting effects.

Precautionary statements:

P261 Avoid breathing dust/ fume/ gas/ mist/ vapours/ spray.

P273 Avoid release to the environment.

P280 Wear protective gloves/ protective clothing.

P301 + P310 + P330 IF SWALLOWED: Immediately call a POISON CENTER/doctor. Rinse mouth.

P391 Collect spillage.

P403 + P233 Store in a well-ventilated place. Keep container tightly closed.

1.2 Solvents

Dry diethyl ether, dry tetrahydrofuran and dry toluene were freshly distilled over sodium wire and benzophenone. Dry dichloromethane was freshly distilled over calcium hydride. Dry *N*,*N*-dimethylformamide was distilled over calcium hydride under vacuum and stored over activated molecular sieves (4 Å). Dry DMSO and dry acetonitrile were obtained by drying over activated molecular sieves (4 Å) for one day prior to use. Dry triethylamine was distilled over calcium hydride and stored over activated molecular sieves (4 Å) under an atmosphere of argon and in the dark. Dry diethyl ether, dry tetrahydrofuran, dry dichloromethane, dry toluene and dry acetonitrile were also provided by a MBraun MB-SPS-5 solvent system, equipped with columns packed with activated alumina to remove moisture and activated copper to remove oxygen (column packing depending on the solvent). Dry methanol was purchased from Acros Organics.

1.3 Thin layer chromatography

TLC analysis was carried out on glass plates coated with silica gel (Merck TLC silica gel 60 F_{254}). Visualization was performed by means of fluorescence in a UV cabinet (254 or 366 nm) or by treatment with a potassium permanganate stain solution. Preparative TLC was carried out on glass plates coated with silica gel (Analtech Uniplate silica gel GF UV_{254}, 20 x 20 cm, layer thickness 2000 µm).

1.4 Column chromatography

Column chromatography was performed in glass columns packed with amorphous silica as solid support (70-200 µm particle size, 60 Å pore size). R_f values were determined by TLC using appropriate solvent mixtures. Automated column chromatography was performed on a Grace Reveleris flash chromatography system or Büchi Reveleris X2 flash system using Grace Reveleris silica cartridges (for normal phase chromatography) or Grace Reveleris C18 reversed-phase flash cartridges (for reverse phase chromatography). Detection of the eluting compounds was performed *via* online UV detection at appropriate wavelengths or *via* online evaporative light-scattering detection (ELSD).

1.5 Microwave reactor

Microwave reactions were performed in a CEM Discover microwave reactor, using capped glass tubes of 10 mL.

1.6 Hydrogenator

Hydrogenation reactions were either performed by equipping the reaction flask with a balloon filled with hydrogen gas or by pressurizing the reaction flask with hydrogen gas by a Parker Balston hydrogen generator, model 75-32-220. The reported pressure (bar) is relative to the atmospheric pressure, *i.e*. 0 bar is equal to atmospheric pressure.

1.7 Elemental analysis

Elemental analysis was performed on a PerkinElmer 2400 Series II CHNS/O analyzer.

1.8 Infrared spectroscopy

Infrared spectra were recorded on a PerkinElmer Spectrum BX FTIR spectrometer equipped with an ATR (attenuated total reflectance) accessory on a ZnSe crystal, or on a Shimadzu IRAffinity-1S FTIR spectrophotometer. Compounds were analyzed in neat form and selected absorbances ν_{max} are reported in cm^{-1}.

1.9 Melting points

The melting points of solid compounds were determined on a Wagner & Munz Heizbank System Kofler (Type WME). Reference materials were used for calibration of the apparatus in the operating temperature range (50 to 260 °C).

1.10 LC-MS analysis

LC-MS analysis was performed on an Agilent 1200 series high performance liquid chromatograph equipped with a Supelco Ascentis Express C18 column (L 3 cm x ID 4.6 mm) packed with 2.7 µm fused-core particles (90 Å pore size) and a DAD-UV/VIS detector operating at 221, 255 and 281 nm. Mass spectra were recorded on an Agilent 1100 series LC/MSD VL mass spectrometer with Electrospray Ionization Geometry (ESI 70 eV) using a quadrupole detector.

1.11 Mass spectrometry

Low-resolution mass spectra were recorded using a direct inlet system on an Agilent 1100 series LC/MSD VL mass spectrometer with Electrospray Ionization Geometry (ESI 70 eV) using a quadrupole detector. Depending on the analyte, the mass spectrometer was set for detection of positive or negative ions. High resolution mass spectrometry (HRMS) was performed on an Agilent 6220 TOF mass spectrometer equipped with an ESI/APCI-multimode source.

1.12 Preparative HPLC

Compounds were purified on an Agilent 1100 series high performance liquid chromatograph equipped with a Supelco Ascentis C18 column (L 15 cm x ID 21.2 mm, 5 µm particle size), a UV VWD detector and a fully automatic fraction collector. The flow rate was set at 6 mL/min and a solvent mixture of HPLC-grade water and acetonitrile was used to elute the compounds.

1.13 NMR spectroscopy

^{1}H- and ^{13}C-NMR spectra were recorded on a Jeol Eclipse 300+ FT-NMR spectrometer (300 MHz and 75 MHz, respectively) or on a Bruker Avance III HD spectrometer (400 MHz and 100 MHz, respectively) at 21 °C. ^{19}F-NMR spectra were recorded at 21 °C on a Bruker Avance III HD spectrometer (376 MHz). The compounds were dissolved in deuterated solvents, using tetramethylsilane (TMS) as an internal standard or using the residual solvent peak as an internal reference for ^{1}H-NMR, and $CFCl_3$ was used as an internal standard for ^{19}F-NMR. The assignment of the different peaks was based on 1D-NMR spectra (^{1}H, ^{13}C, ^{19}F, DEPT) and 2D-NMR spectra (^{1}H-^{1}H COSY, HSQC, H2BC, HMBC). δ values are reported in ppm and *J* values in Hz. Peak multiplicities are reported by the following abbreviations: s = singlet, d = doublet, t = triplet, q = quadruplet, m = multiplet, br = broad.

1.14 X-ray diffraction spectroscopy

For the structures of **323** and **327**, X-ray intensity data were collected at 100 K on a Rigaku Oxford Diffraction Supernova Dual Source (Cu at zero) diffractometer equipped with an Atlas CCD detector using ω scans and CuKα (λ = 1.54184 Å) radiation. The images were interpreted and integrated with the program CrysAlisPro[204]. Using Olex2,[205] the structure was solved by direct methods using the ShelXS structure solution program and refined by full-matrix least-squares on F^2 using the ShelXL program package.[206,207] Non-hydrogen atoms were anisotropically refined and the hydrogen atoms in the riding mode and isotropic temperature factors fixed at 1.2 times U(eq) of the parent atoms (1.5 times for hydroxyl groups). For both structures, the N-H hydrogen atoms were located from a difference Fourier electron density map.

For **323** and **327**, the asymmetric unit has chirality at C1 (*S*), C4 (*R*) and C1 (*R*), C4 (*S*), for **323** and **327**, respectively. But obviously, because of the centro-symmetric space groups of both **323** and **327**, also the inverse configurations are present in the crystal structures.

*Crystal data for compound **323***. $C_6H_8N_2O_3$, M = 156.14, monoclinic, space group $P2_1/c$ (No. 14), a = 11.5884(6) Å, b = 4.9484(2) Å, c = 12.3181(6) Å, β = 113.997(6)°, V = 645.32(6) Å^3, Z = 4, T = 100 K, ρ_{calc} = 1.607 g cm^{-3}, μ(Cu-Kα) = 1.117 mm^{-1}, F(000) = 328, 6184 reflections measured, 1277 unique (R_{int} = 0.0468) which were used in all calculations. The final $R1$ was 0.0417 ($I > 2\sigma(I)$) and $wR2$ was 0.1068 (all data).

*Crystal data for compound **327***. $C_9H_{14}N_2O_5$, M = 230.22, triclinic, space group P-1 (No. 2), a

= 8.6171(3) Å, b = 8.7300(4) Å, c = 8.7325(3) Å, α = 115.865(4)°, β = 94.524(3)°, γ = 117.071(4)°, V = 493.80(5) Å^3, Z = 2, T = 100 K, ρ_{calc} = 1.548 g cm^{-3}, μ(Cu-Kα) = 1.087 mm^{-1}, F(000) = 244, 17583 reflections measured, 2000 unique (R_{int} = 0.0331) which were used in all calculations. The final R1 was 0.0336 (I >2σ (I)) and wR2 was 0.0912 (all data).

CCDC numbers 1900567 and 1900568 contain the supplementary crystallographic data for these compounds and can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB2 1EZ, UK; fax: +44-1223-336033; or deposit@ccdc.cam.ac.uk).

2 Synthetic procedures and characterization

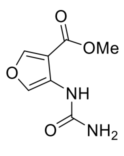
2.1 Furo[3,4-*d*]pyrimidine derivatives

4-(Methoxycarbonyl)furan-3-carboxylic acid (283)

This compound was synthesized in 95% yield according to the method of Hawker and Silverman.[208] All spectra were in accordance with reported data.

Methyl 4-ureidofuran-3-carboxylate (284)

A two-necked, round-bottomed flask of 250 mL is flame dried and equipped with an air cooler and calcium chloride tube. To this flask, 2.409 g of 4-(methoxycarbonyl)furan-3-carboxylic acid **283** (14.2 mmol; 1 eq.), 100 mL of dry toluene and 2.8 mL of triethylamine (1.4 eq.) are added. The reaction is stirred at room temperature until a clear solution is obtained. Then, a solution of 3.2 mL DPPA (1.05 eq.) in 40 mL of dry toluene is added to the reaction and stirred for one hour at room temperature. Then, the temperature is increased to 75 °C and the reaction is stirred for three hours at this temperature. The reaction is cooled down to 0 °C with an ice bath, and a gentle flow of pure ammonia gas is bubbled through the reaction for 5 minutes at 0 °C. The solvent is removed *in vacuo*, and the residue is suspended in 100 mL of water. The pH of this suspension was measured and was around pH = 6. In case the pH is higher than pH = 6, adjust with dilute aqueous hydrochloric acid to pH = 6. The suspension is extracted with 100 mL of ethyl acetate (5x), which turns the suspension into a clear aqueous layer upon extraction of the product by ethyl acetate. The organic layers are combined, dried over magnesium sulfate and filtered. After rotary evaporation of the solvent, 3.43 g of pale yellow product is obtained. The crude product is purified by column chromatography over silica gel, eluting with 20% methanol in ethyl acetate. A pale yellow product is obtained, which is triturated in 10 mL of ethyl acetate. The solvent is removed by filtration and 2.245 g of a white powder is obtained (Y = 86%).



284

^{1}H NMR (400 MHz, DMSO-d$_6$): δ = 3.82 (s, 3H, OC$\underline{H}_3$), 6.51 (br. s, 2H, N$\underline{H}_2$), 7.95 (d, J = 1.8 Hz, 1H, C$\underline{H}_{ar}$), 8.10 (s, 1H, N$\underline{H}$), 8.24 (d, J = 1.8 Hz, 1H, C$\underline{H}_{ar}$). ^{13}C NMR (100 MHz, DMSO-d$_6$): δ = 52.0 (O$\underline{C}$H$_3$), 110.3 ($\underline{C}_q$C(O)OMe), 125.8 ($\underline{C}_q$NHC(O)), 131.4 (O$\underline{C}$HC$_q$NH), 147.5 (O$\underline{C}$HC$_q$C(O)), 155.8 (NH$\underline{C}$(O)NH$_2$), 164.0 ($\underline{C}$(O)OMe). IR: 3442, 3214, 1716, 1678, 1550, 1511, 1349, 1250, 1153, 1118, 1038, 767 cm^{-1}. MS (ESI, 70 eV): m/z (%) = 185.0 (100, $[M + H]^+$). HRMS: m/z $[M + H]^+$ calcd for $C_7H_9N_2O_4$: 185.0557; found: 185.0557. Mp 239-240 °C (degrad.).

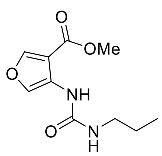
Furo[3,4-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (6)

To a suspension of 1.147 g of sodium methoxide (2.9 eq.) in 26 mL of dry methanol is added 1.348 g of methyl 4-ureidofuran-3-carboxylate **284** (7.3 mmol; 1 eq.). The reaction is stirred at room temperature for three days. After removal of the solvent *in vacuo*, the residue is taken up in 25 mL of water, cooled down to 0 °C and acidified to pH = 5~6 by dropwise addition of 4N aqueous HCl. The white precipitate is filtered off, washed with water (2 x 5 mL) and dried under vacuum to obtain 911 mg of a white powder (Y = 82%). All spectra were in accordance with reported data.[33]

Representative procedure for the synthesis of compounds **36:**

Methyl 4-(3-propylureido)furan-3-carboxylate (36a)

In a flame-dried round-bottomed two-neck flask equipped with an air cooler and $CaCl_2$-tube, DPPA (2.27 ml, 10.5 mmol, 1.05 eq.) was dissolved in dry toluene (100 mL). In another flame-dried round-bottomed flask, 4-(methoxycarbonyl)furan-3-carboxylic acid **283** (1.7 g, 10 mmol, 1 eq.) and dry Et_3N (2 mL, 14 mmol, 1.4 eq.) were dissolved in dry toluene (50 mL). This solution was added to the reaction mixture and stirring was continued at room temperature for 1 hour. Next, the reaction mixture was heated at 75 °C for 3 hours. After cooling to room temperature, *n*-propylamine (1.65 mL, 20 mmol, 2 eq.) was added and the reaction mixture was stirred at room temperature overnight. After evaporation of the solvent, the residue was taken up in EtOAc (100 mL) and was added in a separation funnel together with water (100 mL). While frequently shaking, the aqueous layer was neutralized with dilute HCl (3N). After separation, the aqueous layer was extracted again with EtOAc (100 mL). The combined organic extracts were washed with brine (100 mL), dried over magnesium sulfate and evaporated *in vacuo*. After column chromatography over silica (100% ethyl acetate), 2.26 g of partially purified **36a** was obtained (95% purity, HPLC). For characterization purposes, 500 mg of this compound was used for recrystallization from ethyl acetate to give the title compound **36a** (350 mg, 70%) as white crystals.

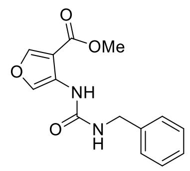


36a

^1H NMR (300 MHz, $CDCl_3$): δ = 0.95 (t, J = 7.3 Hz, 3H), 1.57 (sextet, J = 7.3 Hz, 2H), 3.15-3.27 (m, 2H), 3.84 (s, 3H), 5.33 (br. s, 1H), 7.83 (s, 1H), 8.01 (br. s, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 11.4, 23.3, 42.6, 51.7, 109.9, 125.5, 131.7, 146.2, 155.0, 165.1. IR: 1725, 1648, 1552, 1253, 1154, 766 cm^{-1}. MS (ESI, 70 eV): m/z (%) = 227 (100) $[M + H]^+$. HRMS: m/z $[M + H]^+$ calcd for $C_{10}H_{14}N_2O_4$: 227.1032; found: 227.1029. Mp 141-142 °C; R_f = 0.6 (EtOAc).

Methyl 4-(3-benzylureido)furan-3-carboxylate (36b)

DPPA (1.34 mL, 6.2 mmol, 1.05 eq.) in dry toluene (60 mL) and a solution of 4-(methoxycarbonyl)furan-3-carboxylic acid **283** (1.0 g, 5.9 mmol, 1 eq.) and dry Et_3N (1.17 mL, 8.4 mmol, 1.4 eq.) in dry toluene (30 mL) and benzylamine (1.3 mL, 11.9 mmol, 2 eq.) were used following the representative procedure. After work-up and chromatography, 1.37 g of partially purified **36b** was obtained (95% purity, HPLC). For characterization purposes, 500 mg of this compound was used for recrystallization from EtOH/H_2O (1:1) to give the title compound **36b** (365 mg, 62%) as white crystals.

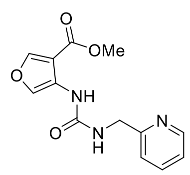


36b

^{1}H NMR (300 MHz, $CDCl_3$): δ = 3.80 (s, 3H), 4.44 (d, J = 5.5 Hz, 2H), 5.25 (br. s, 1H), 7.22-7.38 (m, 5H), 7.81 (s, 1H), 7.98 (s, 1H), 8.00 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 44.8, 51.7, 109.9, 125.4, 127.6, 127.7, 128.8, 131.8, 138.6, 146.2, 154.6, 165.1. IR: 1722, 1646, 1568, 1557, 1304, 1272, 1150, 1086, 766, 696, 679 cm^{-1}. MS (ESI, 70 eV): m/z (%) = 275 (100) $[M + H]^+$. HRMS: m/z $[M + H]^+$ calcd for $C_{14}H_{14}N_2O_4$: 275.1032; found: 275.1032. Mp 113-114 °C; R_f = 0.3 (hexanes-EtOAc, 3:1).

Methyl 4-[3-(pyridin-2-ylmethyl)ureido]furan-3-carboxylate (36c)

DPPA (1.34 mL, 6.2 mmol, 1.05 eq.) in dry toluene (60 mL) and a solution of 4-(methoxycarbonyl)furan-3-carboxylic acid **283** (1.0 g, 5.9 mmol, 1 eq.) and dry Et_3N (1.17 mL, 8.4 mmol, 1.4 eq.) in dry toluene (30 mL) and 2-picolylamine (1.22 mL, 11.8 mmol, 2 eq.) were used following the representative procedure. After work-up and chromatography, 1.29 g of partially purified **36c** was obtained (85% purity, HPLC). For characterization purposes, 500 mg of this compound was used for recrystallization from EtOH to give the title compound **36c** (364 mg, 58%) as off-white crystals.



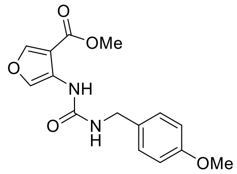
36c

^{1}H NMR (300 MHz, $CDCl_3$): δ = 3.81 (s, 3H), 4.59 (d, J = 5.0 Hz, 2H), 6.32 (br. s, 1H), 7.16-7.24 (m, 1H), 7.31 (d, J = 7.7 Hz, 1H), 7.62-7.72 (m, 1H), 7.82 (s, 1H), 8.02 (s, 1H), 8.07 (s, 1H), 8.55 (d, J = 3.9 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 45.45, 51.7, 109.95, 122.0, 122.5, 125.5, 131.7, 136.9, 146.2, 149.1, 154.7, 157.1, 164.95. IR: 1726, 1649, 1552, 1508, 1250, 1151, 1096, 1085, 1048, 761, 657 cm^{-1}. MS (ESI, 70 eV): m/z (%) = 276 (100) $[M + H]^+$. HRMS: m/z $[M + H]^+$ calcd for $C_{13}H_{13}N_3O_4$: 276.0984; found: 276.0984. Mp 133-134 °C; R_f = 0.22 (hexanes-EtOAc, 1:2).

Methyl 4-[3-(4-methoxybenzyl)ureido]furan-3-carboxylate (36d)

DPPA (1.34 mL, 6.2 mmol, 1.05 eq.) in dry toluene (60 mL) and a solution of 4-(methoxycarbonyl)furan-3-carboxylic acid **283** (1.0 g, 5.9 mmol, 1 eq.) and dry Et_3N (1.17 mL, 8.4 mmol, 1.4 eq.) in dry toluene (30 mL) and 4-methoxybenzylamine (1.54 mL, 11.8 mmol, 2 eq.) were used following the representative procedure. After work-up and chromatography, 1.60 g of partially purified **36d** was obtained (95% purity, HPLC). For characterization purposes, 500 mg of this compound could be recrystallized from EtOH to give the title compound **36d** (358 mg, 64%) as white crystals.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 3.79 (s, 6H), 4.36 (d, J = 5.5 Hz, 2H), 5.28-5.38 (m, 1H), 6.85 (d, J = 8.6 Hz, 2H), 7.23 (d, J = 8.6 Hz, 2H), 7.81 (s, 1H), 7.97 (s, 1H), 8.00 (s, 1H). ^{13}C NMR (75 MHz,



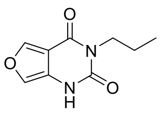
36d

$CDCl_3$): δ = 44.2, 51.7, 55.4, 109.9, 114.1, 125.4, 129.0, 130.7, 131.8, 146.2, 154.6, 159.1, 165.1. IR: 1715, 1644, 1566, 1513, 1242, 1152, 1083, 1034, 768 cm^{-1}. MS (ESI, 70 eV): m/z (%) = 305 (100) $[M + H]^+$. HRMS: m/z $[M + H]^+$ calcd for $C_{15}H_{16}N_2O_5$: 305.1138; found: 305.1139. Mp 139-140 °C; R_f = 0.27 (hexanes-EtOAc, 2:1).

Representative procedure for the synthesis of compounds **38**:

3-Propylfuro[3,4-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (38a)

A solution of **36a** (1.76 g, 7.8 mmol, 1 eq.) in dry THF (40 mL) was cooled down to 0 °C with an ice bath. Next, potassium tert-butoxide (960 mg, 8.6 mmol, 1.1 eq.) was added in one portion and stirring continued at 0 °C for 40 minutes. After quenching the reaction with water (1 mL), the reaction mixture was brought into a separation funnel and EtOAc (100 mL) and water (100 mL) were added. While shaking frequently, the aqueous layer was neutralized with dilute HCl (3N). After separation, the aqueous layer was extracted again with EtOAc (100 mL). The combined organic layers were washed with brine (100 mL), dried over magnesium sulfate and evaporated *in vacuo*. The crude product was recrystallized from MeOH to give the title compound **38a** (1.33 g, 88% over two steps) as white crystals.

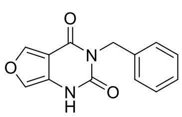


38a

^{1}H NMR (300 MHz, DMSO-d6): δ = 0.84 (t, J = 7.5 Hz, 3H, $C\underline{H}_3$), 1.52 (sextet, J = 7.5 Hz, 2H, $C\underline{H}_2CH_3$), 3.74 (t, J = 7.5 Hz, 2H, $NC\underline{H}_2$), 7.61 (s, 1H, $OC\underline{H}C_qNH$), 8.43 (s, 1H, $OC\underline{H}C_qC(O)$), 10.87 (s, 1H, $N\underline{H}$). ^{13}C NMR (75 MHz, DMSO-d6): δ = 11.7 ($\underline{C}H_3$), 21.5 ($\underline{C}H_2CH_3$), 41.7 ($N\underline{C}H_2$), 110.9 ($\underline{C}_qC(O)$), 125.5 ($\underline{C}_qNHC(O)$), 125.9 ($O\underline{C}HC_qNH$), 145.0 ($O\underline{C}HC_qC(O)$), 151.2 ($NH\underline{C}(O)N$), 159.0 ($C_q\underline{C}(O)N$). IR: 1723, 1652, 1345, 1241, 746, 698 cm^{-1}. MS (ESI, 70 eV): m/z (%) = 193 (100) $[M-H]^-$. HRMS: m/z $[M - H]^-$ calcd for $C_9H_{10}N_2O_3$: 193.0613; found: 193.0623. Mp 163-164 °C (degrad.).

3-Benzylfuro[3,4-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (38b)

36b (0.87 g, 3.2 mmol, 1 eq.) in dry THF (20 mL) and potassium tert-butoxide (392 mg, 3.5 mmol, 1.1 eq.) were used following the representative procedure. The crude product was recrystallized from MeOH to give the title compound **38b** (696 mg, 77% over two steps) as white crystals.

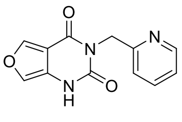


38b

^{1}H NMR (300 MHz, DMSO-d6): δ = 4.99 (s, 2H), 7.18-7.34 (m, 5H), 7.66 (s, 1H), 8.49 (s, 1H), 11.01 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d6): δ = 43.3, 110.8, 125.5, 126.2, 127.6, 127.9, 128.9, 138.2, 145.3, 151.3, 159.1. IR: 1725, 1652, 1345, 1240, 751, 699, 683 cm^{-1}. MS (ESI, 70 eV): m/z (%) = 241 (100) $[M-H]^-$. HRMS: m/z $[M - H]^-$ calcd for $C_{13}H_{10}N_2O_3$: 241.0613; found: 241.0623. Mp 241-242 °C (degrad.).

3-(Pyridin-2-ylmethyl)furo[3,4-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (38c)

36c (0.79 g, 2.9 mmol, 1 eq.) in dry THF (20 mL) and potassium *tert*-butoxide (354 mg, 3.2 mmol, 1.1 eq.) were used following the representative procedure. The crude product was recrystallized from EtOH to give the title compound **38c** (578 mg, 66% over two steps) as white crystals.

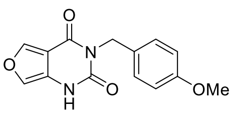


38c

^{1}H NMR (300 MHz, DMSO-d6): δ = 5.11 (s, 2H), 7.18-7.29 (m, 2H), 7.66-7.76 (m, 2H), 8.42 (d, J = 4.5 Hz, 1H), 8.49 (s, 1H), 10.99 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d6): δ = 44.7, 110.8, 121.2, 122.6, 125.6, 126.2, 137.2, 145.3, 149.4, 151.3, 156.8, 159.1. IR: 1713, 1668, 1648, 1386, 1329, 1244, 765, 694 cm^{-1}. MS (ESI, 70 eV): m/z (%) = 244 (100) [M+H]$^+$. HRMS: m/z [M + H]$^+$ calcd for $C_{12}H_9N_3O_3$: 244.0722; found: 244.0718. Mp 228-229 °C (degrad.).

3-(4-Methoxybenzyl)furo[3,4-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (38d)

36d (1.10 g, 3.6 mmol, 1 eq.) in dry THF (20 mL) and potassium tert-butoxide (446 mg, 4.0 mmol, 1.1 eq.) were used following the representative procedure. The crude product was recrystallized from MeOH to give the title compound **38d** (836 mg, 76% over two steps) as white crystals.

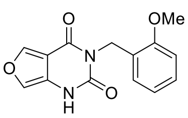


38d

^{1}H NMR (300 MHz, DMSO-d6): δ = 3.69 (s, 3H), 4.91 (s, 2H), 6.84 (d, J = 8.4 Hz, 2H), 7.23 (d, J = 8.4 Hz, 2H), 7.64 (s, 1H), 8.47 (s, 1H), 10.97 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d6): δ = 42.7, 55.6, 110.8, 114.2, 125.4, 126.1, 129.7, 130.3, 145.2, 151.3, 158.9, 159.0. IR: 1721, 1661, 1652, 1242, 1028, 766, 750, 721 cm^{-1}. MS (ESI, 70 eV): m/z (%) = 271 (100) [M-H]$^-$. HRMS: m/z [M - H]$^-$ calcd for $C_{14}H_{12}N_2O_4$: 271.0719; found: 271.0731. Mp 217-218 °C (degrad).

3-(2-Methoxybenzyl)furo[3,4-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (38e)

DPPA (0.13 mL, 0.62 mmol, 1.05 eq.) in dry toluene (6 mL) and a solution of 4-(methoxycarbonyl)furan-3-carboxylic acid **283** (100 mg, 0.59 mmol, 1 eq.) and dry Et_3N (0.12 mL, 0.84 mmol, 1.4 eq.) in dry toluene (3 mL) and 2-methoxybenzylamine (0.16 mL, 1.18 mmol, 2 eq.) were used following the representative procedure for compound **36**. After work-up and chromatography (hexanes-EtOAc, 4:1, R_f = 0.17), 163 mg of **36e** was obtained. Next, **36e** (163 mg, 0.54 mmol, 1 eq.) in dry THF (2 mL) and potassium tert-butoxide (66 mg, 0.59 mmol, 1.1 eq.) were used following the representative procedure for compound **38**. The crude product was recrystallized from MeOH to give the title compound **38e** (126 mg, 79% over two steps) as white crystals.

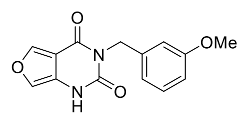


38e

^{1}H NMR (400 MHz, DMSO-d6): δ = 3.85 (s, 3H), 4.96 (s, 2H), 6.78 (d, J = 7.6 Hz, 1H), 6.85 (t, J = 7.6 Hz, 1H), 7.01 (d, J = 7.6 Hz, 1H), 7.22 (t, J = 7.6 Hz, 1H), 7.70 (s, 1H), 8.51 (s, 1H), 11.03 (br. s, 1H). ^{13}C NMR (100 MHz, DMSO-d6): δ = 38.9, 55.8, 110.7, 110.9, 120.65, 125.3, 125.4, 125.5, 126.1, 128.15, 145.2, 151.1, 156.8, 159.0. IR: 1724, 1660, 1240, 1027, 743 cm^{-1}. MS (ESI, 70 eV): m/z (%) = 273 (100) [M+H]$^+$. HRMS: m/z [M + H]$^+$ calcd for $C_{14}H_{12}N_2O_4$: 273.0870; found: 273.0882. Mp 209-210 °C (degrad.).

3-(3-Methoxybenzyl)furo[3,4-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (38f)

DPPA (0.13 mL, 0.62 mmol, 1.05 eq.) in dry toluene (6 mL) and a solution of **283** (100 mg, 0.59 mmol, 1 eq.) and dry Et_3N (0.12 mL, 0.84 mmol, 1.4 eq.) in dry toluene (3 mL) and 3-methoxybenzylamine (0.15 mL, 1.18 mmol, 2 eq.) were used following the representative procedure for compound **36**. After work-up and chromatography (hexanes-EtOAc, 3:1, R_f = 0.22), 152 mg of **36f** was obtained. Next, **36f** (152 mg, 0.50 mmol, 1 eq.) in dry THF (2 mL) and potassium tert-butoxide (62 mg, 0.55 mmol, 1.1 eq.) were used following the representative procedure for compound **38**. The crude product was recrystallized from MeOH to give the title compound **38f** (118 mg, 74% over two steps) as white crystals.

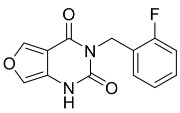


38f

^{1}H NMR (400 MHz, DMSO-d6): δ = 3.72 (s, 3H), 4.98 (s, 2H), 6.79-6.86 (m, 3H), 7.22 (t, J = 8.0 Hz, 1H), 7.67 (d, J = 1.3 Hz, 1H), 8.50 (d, J = 1.3 Hz, 1H), 11.00 (br. s, 1H). ^{13}C NMR (100 MHz, DMSO-d6): δ = 43.1, 55.4, 110.6, 112.7, 113.7, 119.8, 125.35, 126.1, 129.9, 139.7, 145.2, 151.2, 159.0, 159.7. IR: 1731, 1664, 1652, 1389, 1239, 760, 709 cm^{-1}. MS (ESI, 70 eV): m/z (%) = 273 (100) $[M+H]^+$. HRMS: m/z $[M + H]^+$ calcd for $C_{14}H_{12}N_2O_4$: 273.0870; found: 273.0859. Mp 187-188 °C (degrad.).

3-(2-Fluorobenzyl)furo[3,4-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (38g)

DPPA (0.13 mL, 0.62 mmol, 1.05 eq.) in dry toluene (6 mL) and a solution of **283** (100 mg, 0.59 mmol, 1 eq.) and dry Et_3N (0.12 mL, 0.84 mmol, 1.4 eq.) in dry toluene (3 mL) and 2-fluorobenzylamine (0.14 mL, 1.18 mmol, 2 eq.) were used following the representative procedure for compound **36**. After work-up and chromatography (hexanes-EtOAc, 5:1, R_f = 0.22), 160 mg of **36g** was obtained. Next, **36g** (160 mg, 0.55 mmol, 1 eq.) in dry THF (2 mL) and potassium tert-butoxide (68 mg, 0.60 mmol, 1.1 eq.) were used following the representative procedure for compound **38**. The crude product was recrystallized from MeOH to give the title compound **38g** (115 mg, 75% over two steps) as white crystals.

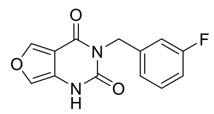


38g

^{1}H NMR (400 MHz, DMSO-d6): δ = 5.06 (s, 2H), 7.01-7.40 (m, 4H), 7.69 (s, 1H), 8.52 (s, 1H), 11.05 (br. s, 1H). ^{13}C NMR (100 MHz, DMSO-d6): δ = 37.3 (d, J = 5.1 Hz), 110.6, 115.6 (d, J = 21.1 Hz), 124.8 (d, J = 14.0 Hz), 124.9 (d, J = 3.2 Hz), 125.4, 126.2, 128.5 (d, J = 4.1 Hz), 129.3 (d, J = 8.1 Hz), 145.3, 151.0, 158.9, 160.3 (d, J = 244.4 Hz). ^{19}F NMR (376 MHz, DMSO-d6): δ = -118.3. IR: 1729, 1660, 1334, 1243, 1230, 753, 718 cm^{-1}. MS (ESI, 70 eV): m/z (%) = 261 (100) $[M+H]^+$. HRMS: m/z $[M + H]^+$ calcd for $C_{13}H_9FN_2O_3$: 261.0670; found: 261.0665. Mp 244-245 °C (degrad.).

3-(3-Fluorobenzyl)furo[3,4-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (38h)

DPPA (0.13 mL, 0.62 mmol, 1.05 eq.) in dry toluene (6 mL) and a solution of **283** (100 mg, 0.59 mmol, 1 eq.) and dry Et_3N (0.12 mL, 0.84 mmol, 1.4 eq.) in dry toluene (3 mL) and 3-fluorobenzylamine (0.14 mL, 1.18 mmol, 2 eq.) were used following the representative procedure for compound **36**. After work-up and chromatography (hexanes-EtOAc, 5:1, R_f = 0.22), 149 mg of **36h** was obtained. Next, **36h** (149 mg, 0.51 mmol, 1 eq.) in dry THF (2 mL) and potassium tert-butoxide (63 mg, 0.56 mmol, 1.1 eq.) were used following the representative procedure for compound **38**. The crude product was recrystallized from MeOH to give the title compound **38h** (109 mg, 71% over two steps) as white crystals.

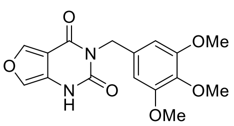


38h

^{1}H NMR (400 MHz, DMSO-d6): δ = 5.01 (s, 2H), 7.04-7.15 (m, 3H), 7.31-7.40 (m, 1H), 7.68 (d, J = 1.6 Hz, 1H), 8.51 (d, J = 1.6 Hz, 1H), 11.03 (br. s, 1H). ^{13}C NMR (100 MHz, DMSO-d6): δ = 42.8, 110.6, 114.3 (d, J = 20.8 Hz), 114.6 (d, J = 21.9 Hz), 123.7 (d, J = 2.6 Hz), 125.4, 126.1, 130.8 (d, J = 8.5 Hz), 141.0 (d, J = 7.3 Hz), 145.3, 151.1, 159.0, 162.6 (d, J = 243.5 Hz). ^{19}F NMR (376 MHz, DMSO-d6): δ = -113.0. IR: 1724, 1652, 1336, 1275, 1237, 767, 739 cm^{-1}. MS (ESI, 70 eV): m/z (%) = 259 (100) [M-H]$^{-}$. HRMS: m/z [M + H]$^{+}$ calcd for $C_{13}H_{9}FN_{2}O_{3}$: 261.0670; found: 261.0678. Mp 220-221 °C (degrad.).

3-(3,4,5-Trimethoxybenzyl)furo[3,4-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (38i)

DPPA (0.13 mL, 0.62 mmol, 1.05 eq.) in dry toluene (6 mL) and a solution of **283** (100 mg, 0.59 mmol, 1 eq.) and dry $Et_{3}N$ (0.12 mL, 0.84 mmol, 1.4 eq.) in dry toluene (3 mL) and 3,4,5-trimethoxybenzylamine (0.20 mL, 1.18 mmol, 2 eq.) were used following the representative procedure for compound **36**. After work-up and chromatography (hexanes-EtOAc, 2:1, R_f = 0.17), 197 mg of **36i** was obtained. Next, **36i** (197 mg, 0.54 mmol, 1 eq.) in dry THF (2 mL) and potassium tert-butoxide (67 mg, 0.59 mmol, 1.1 eq.) were used following the representative procedure for compound **38**. The crude product was recrystallized from MeOH to give the title compound **38i** (158 mg, 81% over two steps) as white crystals.

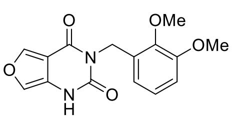


38i

^{1}H NMR (400 MHz, DMSO-d6): δ = 3.62 (s, 3H), 3.72 (s, 6H), 4.94 (s, 2H), 6.61 (s, 2H), 7.67 (d, J = 1.6 Hz, 1H), 8.50 (d, J = 1.6 Hz, 1H), 10.99 (br. s, 1H). ^{13}C NMR (100 MHz, DMSO-d6): δ = 43.4, 56.3, 60.4, 105.6, 110.7, 125.4, 126.1, 133.8, 137.1, 145.2, 151.2, 153.2, 159.0. IR: 1729, 1657, 1591, 1350, 1333, 1236, 1114, 1011, 747, 704 cm^{-1}. MS (ESI, 70 eV): m/z (%) = 333 (100) [M+H]$^{+}$. HRMS: m/z [M + H]$^{+}$ calcd for $C_{16}H_{16}N_{2}O_{6}$: 333.1081; found: 333.1085. Mp 218-219 °C (degrad.).

3-(2,3-Dimethoxybenzyl)furo[3,4-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (38j)

DPPA (0.13 mL, 0.62 mmol, 1.05 eq.) in dry toluene (6 mL) and a solution of **283** (100 mg, 0.59 mmol, 1 eq.) and dry $Et_{3}N$ (0.12 mL, 0.84 mmol, 1.4 eq.) in dry toluene (3 mL) and 2,3-dimethoxybenzylamine (0.18 mL, 1.18 mmol, 2 eq.) were used following the representative procedure for compound **36**. After work-up and chromatography (hexanes-EtOAc, 2:1, R_f = 0.17), 185 mg of **36j** was obtained. Next, **36j** (185 mg, 0.55 mmol, 1 eq.) in dry THF (2 mL) and potassium tert-butoxide (68 mg, 0.61 mmol, 1.1 eq.) were used following the representative procedure for compound **38**. The crude product was recrystallized from MeOH to give the title compound **38j** (137 mg, 77% over two steps) as white crystals.

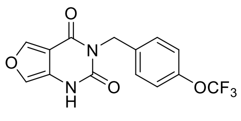


38j

^{1}H NMR (400 MHz, DMSO-d6): δ = 3.80 (s, 3H), 3.81 (s, 3H), 5.03 (s, 2H), 6.49 (d, J = 6.6 Hz, 1H), 6.89-7.02 (m, 2H), 7.69 (s, 1H), 8.50 (s, 1H), 11.01 (br. s, 1H). ^{13}C NMR (100 MHz, DMSO-d6): δ = 38.5, 56.1, 60.2, 110.7, 111.9, 118.3, 124.3, 125.4, 126.1, 131.4, 145.2, 146.4, 151.1, 152.8, 159.0. IR: 1732, 1660, 1334, 1234, 1221, 1066, 758, 744 cm^{-1}. MS (ESI, 70 eV): m/z (%) = 303 (100) $[M+H]^+$. HRMS: m/z $[M + H]^+$ calcd for $C_{15}H_{14}N_2O_5$: 303.09755; found: 303.0974. Mp 215-216 °C (degrad.).

3-[4-(Trifluoromethoxy)benzyl]furo[3,4-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (38k)

DPPA (0.13 mL, 0.62 mmol, 1.05 eq.) in dry toluene (6 mL) and a solution of **283** (100 mg, 0.59 mmol, 1 eq.) and dry Et_3N (0.12 mL, 0.84 mmol, 1.4 eq.) in dry toluene (3 mL) and 4-(trifluoromethoxy)benzylamine (0.18 mL, 1.18 mmol, 2 eq.) were used following the representative procedure for compound **36**. After work-up and chromatography (hexanes-EtOAc, 4:1, R_f = 0.21), 206 mg of **36k** was obtained. Next, **36k** (206 mg, 0.57 mmol, 1 eq.) in dry THF (2 mL) and potassium tert-butoxide (71 mg, 0.63 mmol, 1.1 eq.) were used following the representative procedure for compound **38**. The crude product was recrystallized from MeOH to give the title compound **38k** (157 mg, 82% over two steps) as white crystals.

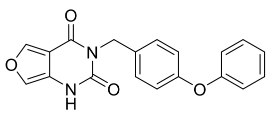


38k

^{1}H NMR (400 MHz, DMSO-d6): δ = 5.03 (s, 2H), 7.31 (d, J = 8.4 Hz, 2H), 7.42 (d, J = 8.4 Hz, 2H), 7.68 (d, J = 1.6 Hz, 1H), 8.51 (d, J = 1.6 Hz, 1H), 11.04 (br. s, 1H). ^{13}C NMR (100 MHz, DMSO-d6): δ = 42.6, 110.6, 120.5 (q, J = 255.9 Hz), 121.4, 125.4, 126.2, 129.8, 137.6, 145.3, 147.7, 151.1, 159.0. ^{19}F NMR (376 MHz, DMSO-d6): δ = -56.4. IR: 1728, 1667, 1281, 1239, 1211, 1146, 1098, 706 cm^{-1}. MS (ESI, 70 eV): m/z (%) = 325 (100) $[M-H]^-$. HRMS: m/z $[M - H]^-$ calcd for $C_{14}H_9F_3N_2O_4$: 325.0442; found: 325.0453. Mp 203-204 °C (degrad.).

3-(4-Phenoxybenzyl)furo[3,4-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (38l)

DPPA (0.13 mL, 0.62 mmol, 1.05 eq.) in dry toluene (6 mL) and a solution of **283** (100 mg, 0.59 mmol, 1 eq.) and dry Et_3N (0.12 mL, 0.84 mmol, 1.4 eq.) in dry toluene (3 mL) and 4-phenoxybenzylamine (0.21 mL, 1.18 mmol, 2 eq.) were used following the representative procedure for compound **36**. After work-up and chromatography (hexanes-EtOAc, 4:1, R_f = 0.27), 205 mg of **36l** was obtained. Next, **36l** (205 mg, 0.56 mmol, 1 eq.) in dry THF (2 mL) and potassium tert-butoxide (69 mg, 0.62 mmol, 1.1 eq.) were used following the representative procedure for compound **38**. The crude product was recrystallized from MeOH to give the title compound **38l** (157 mg, 80% over two steps) as white crystals.

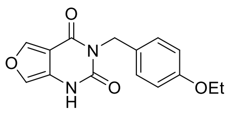


38l

^{1}H NMR (400 MHz, DMSO-d6): δ = 4.99 (s, 2H), 6.80-7.50 (m, 9H), 7.67 (s, 1H), 8.50 (s, 1H), 11.01 (br. s, 1H). ^{13}C NMR (100 MHz, DMSO-d6): δ = 42.6, 110.7, 118.9, 119.0, 123.8, 125.3, 126.1, 129.9, 130.5, 133.3, 145.2, 151.2, 156.1, 157.2, 158.9. IR: 1727, 1651, 1343, 1236, 752, 738, 690 cm^{-1}. MS (ESI, 70 eV): m/z (%) = 335 (100) $[M+H]^+$. HRMS: m/z $[M + H]^+$ calcd for $C_{19}H_{14}N_2O_4$: 335.1026; found: 335.1027. Mp 209-210 °C (degrad.).

3-(4-Ethoxybenzyl)furo[3,4-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (38m)

DPPA (0.13 mL, 0.62 mmol, 1.05 eq.) in dry toluene (6 mL) and a solution of **283** (100 mg, 0.59 mmol, 1 eq.) and dry Et_3N (0.12 mL, 0.84 mmol, 1.4 eq.) in dry toluene (3 mL) and 4-ethoxybenzylamine (178 mg, 1.18 mmol, 2 eq.) were used following the representative procedure for compound **36**. After work-up and chromatography (hexanes-EtOAc, 4:1, R_f = 0.25), 167 mg of **36m** was obtained. Next, **36m** (167 mg, 0.52 mmol, 1 eq.) in dry THF (2 mL) and potassium tert-butoxide (65 mg, 0.58 mmol, 1.1 eq.) were used following the representative procedure for compound **38**. The crude product was recrystallized from MeOH to give the title compound **38m** (128 mg, 76% over two steps) as white crystals.

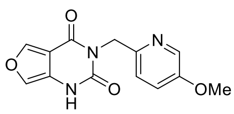


38m

^{1}H NMR (400 MHz, DMSO-d6): δ = 1.30 (t, J = 6.9 Hz, 3H), 3.98 (q, J = 6.9 Hz, 2H), 4.93 (s, 2H), 6.85 (d, J = 8.4 Hz, 2H), 7.23 (d, J = 8.4 Hz, 2H), 7.65 (s, 1H), 8.48 (s, 1H), 10.97 (br. s, 1H). ^{13}C NMR (100 MHz, DMSO-d6): δ = 15.1, 42.6, 63.4, 110.7, 114.6, 125.3, 126.0, 129.6, 130.0, 145.1, 151.2, 158.1, 158.9. IR: 1729, 1659, 1250, 1236, 1220, 744, 681 cm^{-1}. MS (ESI, 70 eV): m/z (%) = 287 (100) $[M+H]^+$. HRMS: m/z $[M + H]^+$ calcd for $C_{15}H_{14}N_2O_4$: 287.1026; found: 287.1033. Mp 209-210 °C (degrad.).

3-[(6-Methoxypyridin-3-yl)methyl]furo[3,4-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (38n)

DPPA (0.13 mL, 0.62 mmol, 1.05 eq.) in dry toluene (6 mL) and a solution of **283** (100 mg, 0.59 mmol, 1 eq.) and dry Et_3N (0.12 mL, 0.84 mmol, 1.4 eq.) in dry toluene (3 mL) and 5-(aminomethyl)-2-methoxypyridine (162 mg, 1.18 mmol, 2 eq.) were used following the representative procedure for compound **36**. After work-up and chromatography (hexanes-EtOAc, 2:1, R_f = 0.19), 147 mg of **36n** was obtained. Next, **36n** (147 mg, 0.48 mmol, 1 eq.) in dry THF (2 mL) and potassium tert-butoxide (59 mg, 0.53 mmol, 1.1 eq.) were used following the representative procedure for compound **38**. The crude product was recrystallized from MeOH to give the title compound **38n** (111 mg, 69% over two steps) as white crystals.

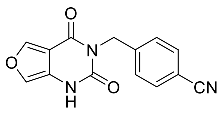


38n

^{1}H NMR (400 MHz, DMSO-d6): δ = 3.82 (s, 3H), 4.94 (s, 2H), 6.76 (d, J = 8.5 Hz, 1H), 7.63-7.68 (m, 2H), 8.15 (d, J = 2.1 Hz, 1H), 8.49 (d, J = 1.6 Hz, 1H), 11.01 (br. s, 1H). ^{13}C NMR (100 MHz, DMSO-d6): δ = 40.4, 53.6, 110.66, 110.67, 125.3, 126.1, 126.85, 139.7, 145.2, 147.1, 151.1, 158.95, 163.3. IR: 1720, 1673, 1499, 1394, 1330, 1293, 1243, 1028, 1017, 873, 760, 743, 688 cm^{-1}. MS (ESI, 70 eV): m/z (%) = 274 (100) $[M+H]^+$. HRMS: m/z $[M + H]^+$ calcd for $C_{13}H_{11}N_3O_4$: 274.0822; found: 274.0828. Mp 211-212 °C (degrad.).

3-(4-Cyanobenzyl)furo[3,4-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (38o)

DPPA (0.13 mL, 0.62 mmol, 1.05 eq.) in dry toluene (6 mL) and a solution of **283** (100 mg, 0.59 mmol, 1 eq.) and dry Et_3N (0.12 mL, 0.84 mmol, 1.4 eq.) in dry toluene (3 mL) and 4-cyanobenzylamine (155 mg, 1.18 mmol, 2 eq.) were used following the representative procedure for compound **36**. After work-up and chromatography (hexanes-EtOAc, 2:1, R_f = 0.24), 162 mg of **36o** was obtained. Next, **36o** (162 mg, 0.54 mmol, 1 eq.) in dry THF (2 mL) and potassium tert-butoxide (67 mg, 0.60 mmol, 1.1 eq.) were used following the representative procedure for compound **38**. The crude product was recrystallized from MeOH to give the title compound **38o** (123 mg, 78% over two steps) as white crystals.



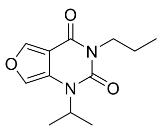
38o

^{1}H NMR (400 MHz, DMSO-d6): δ = 5.08 (s, 2H), 7.46 (d, J = 8.0 Hz, 2H), 7.69 (s, 1H), 7.78 (d, J = 8.0 Hz, 2H), 8.52 (s, 1H), 11.07 (br. s, 1H). ^{13}C NMR (100 MHz, DMSO-d6): δ = 43.2, 110.2, 110.6, 119.3, 125.4, 126.2, 128.5, 132.8, 143.9, 145.3, 151.1, 159.0. IR: 2226, 1727, 1662, 1431, 1340, 1235, 760, 740, 712 cm^{-1}. MS (ESI, 70 eV): m/z (%) = 266 (100) [M-H]$^-$. HRMS: m/z [M - H]$^-$ calcd for $C_{14}H_9N_3O_3$: 266.0571; found: 266.0573. Mp 250-251 °C (degrad.).

Representative procedure for the synthesis of **285**:

1-Isopropyl-3-propylfuro[3,4-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (285a)

To a solution of **38a** (500 mg, 2.57 mmol, 1 eq.) in dry DMF (20 mL), Cs_2CO_3 (2.52 g, 7.7 mmol, 3 eq.) and TBAI (2.85 g, 7.7 mmol, 3 eq.) were added under an inert atmosphere. After stirring the reaction for 30 minutes at room temperature, 2-bromopropane (0.73 ml, 7.7 mmol, 3 eq.) was added and stirring continued at room temperature for 24 hours. Water (50 mL) was added, and the reaction mixture was extracted with EtOAc (3x 50 mL). The combined organic layers were combined, washed with brine (50 mL), dried over $MgSO_4$ and evaporated *in vacuo.* The crude product was purified by column chromatography over silica and subsequently recrystallized from EtOH to give the title compound **285a** (335 mg, 55%) as white crystals.

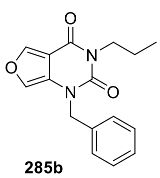


285a

^{1}H NMR (300 MHz, DMSO-d6): δ = 0.83 (t, J = 7.6 Hz, 3H, $CH_2C\underline{H_3}$), 1.35 (d, J = 7.1 Hz, 6H, $CH(C\underline{H_3})_2$), 1.51 (sextet, J = 7.6 Hz, 2H, $C\underline{H_2}CH_3$), 3.76 (t, J = 7.6 Hz, 2H, $NC\underline{H_2}$), 4.75 (septet, J = 7.1 Hz, 1H, $C\underline{H}(CH_3)_2$), 8.06 (d, J = 1.4 Hz, 1H, $OC\underline{H}C_qN$), 8.51 (d, J = 1.4 Hz, 1H, $OC\underline{H}C_qC(O)$). ^{13}C NMR (75 MHz, DMSO-d6): δ = 11.7 ($CH_2\underline{C}H_3$), 18.8 ($CH(\underline{C}H_3)_2$), 21.5 ($\underline{C}H_2CH_3$), 42.5 ($N\underline{C}H_2$), 48.9 ($\underline{C}H(CH_3)_2$), 111.5 ($\underline{C_q}C(O)$), 125.4 ($\underline{C_q}NC(O)$), 126.9 ($O\underline{C}HC_qN$), 145.2 ($O\underline{C}HC_qC(O)$), 150.4 ($N\underline{C}(O)N$), 158.2 ($C_q\underline{C}(O)N$). IR: 1701, 1660, 1630, 1259, 1068, 870, 757, 748 cm^{-1}. MS (ESI, 70 eV): m/z (%) = 237 (100) [M+H]$^+$. HRMS: m/z [M + H]$^+$ calcd for $C_{12}H_{16}N_2O_3$: 237.1239; found: 237.1232. Mp 55-56 °C; R_f = 0.15 (hexanes-EtOAc, 5:1).

1-Benzyl-3-propylfuro[3,4-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (285b)

38a (500 mg, 2.57 mmol, 1 eq.) in dry DMF (20 mL), Cs_2CO_3 (2.52 g, 7.7 mmol, 3 eq.), TBAI (2.85 g, 7.7 mmol, 3 eq.) and benzyl bromide (0.92 ml, 7.7 mmol, 3 eq.) were used following the representative procedure for compound **285**. After work-up and chromatography over silica, the title compound **285b** (731 mg, 99%) was obtained as a white solid.

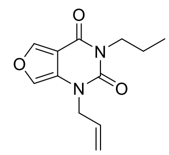


285b

^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.98 (t, J = 7.4 Hz, 3H), 1.70 (sextet, J = 7.4 Hz, 2H), 3.99 (t, J = 7.4 Hz, 2H), 5.00 (s, 2H), 7.17 (d, J = 1.7 Hz, 1H), 7.25-7.39 (m, 5H), 8.02 (d, J = 1.7 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 11.4, 21.5, 43.1, 49.5, 111.2, 125.1, 127.6, 127.9, 128.15, 129.0, 135.3, 144.1, 151.4, 158.4. IR: 1709, 1662, 1638, 1392, 1334, 1251, 1035, 746, 711, 697 cm^{-1}. MS (ESI, 70 eV): m/z (%) = 285 (100) $[M+H]^+$. HRMS: m/z $[M + H]^+$ calcd for $C_{16}H_{16}N_2O_3$: 285.1239; found: 285.1232. Mp 118-119 °C; R_f = 0.22 (hexanes-EtOAc, 5:1).

1-Allyl-3-propylfuro[3,4-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (285c)

38a (500 mg, 2.57 mmol, 1 eq.) in dry DMF (20 mL), Cs_2CO_3 (2.52 g, 7.7 mmol, 3 eq.), TBAI (2.85 g, 7.7 mmol, 3 eq.) and allyl bromide (0.67 ml, 7.7 mmol, 3 eq.) were used following the representative procedure for compound **285**. After work-up, chromatography over silica and subsequent recrystallization from EtOH, the title compound **285c** (307 mg, 51%) was obtained as white crystals.

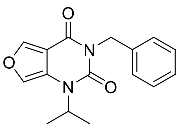


285c

^{1}H NMR (300 MHz, DMSO-d6): δ = 0.84 (t, J = 7.4 Hz, 3H), 1.53 (sextet, J = 7.4 Hz, 2H), 3.79 (t, J = 7.4 Hz, 2H), 4.35 (d, J = 5.1 Hz, 2H), 5.13-5.30 (m, 2H), 5.75-5.90 (m, 1H), 7.87 (d, J = 1.7 Hz, 1H), 8.51 (d, J = 1.7 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d6): δ = 11.7, 21.5, 42.6, 48.1, 111.0, 118.3, 126.7, 127.8, 131.8, 145.6, 150.6, 158.4. IR: 1712, 1659, 1636, 1340, 1288, 1266, 764 cm^{-1}. MS (ESI, 70 eV): m/z (%) = 235 (100) $[M+H]^+$. HRMS: m/z $[M + H]^+$ calcd for $C_{12}H_{14}N_2O_3$: 235.1083; found: 235.1081. Mp 77-78 °C; R_f = 0.22 (hexanes-EtOAc, 5:1).

3-Benzyl-1-isopropylfuro[3,4-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (285d)

38b (375 mg, 1.55 mmol, 1 eq.) in dry DMF (12 mL), Cs_2CO_3 (1.52 g, 4.65 mmol, 3 eq.), TBAI (1.72 g, 4.65 mmol, 3 eq.) and 2-bromopropane (0.44 ml, 4.65 mmol, 3 eq.) were used following the representative procedure for compound **285**. After work-up, chromatography over silica and subsequent recrystallization from EtOH, the title compound **285d** (290 mg, 66%) was obtained as white crystals.



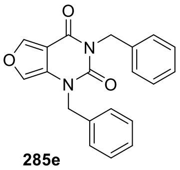
285d

^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.43 (d, J = 6.8 Hz, 6H), 4.93 (septet, J = 6.8 Hz, 1H), 5.16 (s, 2H), 7.21-7.36 (m, 3H), 7.39 (d, J = 1.7 Hz, 1H), 7.45-7.52 (m, 2H), 8.08 (d, J = 1.7 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 18.8, 44.5, 48.8, 111.7, 125.0, 125.25, 127.6, 128.5, 129.0, 137.45, 144.05, 150.7, 158.4. IR: 1704, 1660, 1627, 1347, 1262, 708 cm^{-1}. MS (ESI, 70 eV): m/z (%) = 285 (100) $[M+H]^+$. HRMS: m/z $[M + H]^+$ calcd for $C_{16}H_{16}N_2O_3$: 285.1239; found: 285.1235. Mp 100-101 °C; R_f = 0.24 (hexanes-EtOAc, 5:1).

1,3-Dibenzylfuro[3,4-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (285e)

38b (120 mg, 0.50 mmol, 1 eq.) in dry DMF (5 mL), Cs_2CO_3 (0.49 g, 1.50 mmol, 3 eq.), TBAI (0.55 g, 1.50 mmol, 3 eq.) and benzyl bromide (0.18 ml, 1.50 mmol, 3 eq.) were used following the representative procedure for compound **285**. After work-up, chromatography over silica and subsequent recrystallization from EtOH, the title compound **285e** (141 mg, 86%) was obtained as white crystals.

^{1}H NMR (300 MHz, acetone-d6): δ = 5.05 (s, 2H), 5.17 (s, 2H), 7.18-7.48 (m, 10H), 7.67 (s, 1H), 8.29 (s, 1H). ^{13}C NMR (75 MHz, acetone-d6): δ = 44.0, 48.9, 111.2, 126.1, 127.2, 127.67, 127.73, 128.1, 128.2, 128.3, 128.7, 136.15, 138.05, 144.8, 151.3, 158.0. IR: 1709, 1664, 1638, 1339, 1258, 714, 708

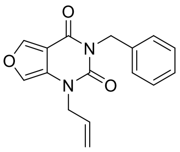


285e

cm^{-1}. MS (ESI, 70 eV): m/z (%) = 333 (100) $[M+H]^+$. HRMS: m/z $[M + H]^+$ calcd for $C_{20}H_{16}N_2O_3$: 333.1239; found: 333.1235. Mp 108-109 °C; R_f = 0.20 (hexanes-EtOAc, 5:1).

1-Allyl-3-benzylfuro[3,4-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (285f)

38b (500 mg, 2.06 mmol, 1 eq.) in dry DMF (20 mL), Cs_2CO_3 (2.0 g, 6.18 mmol, 3 eq.), TBAI (2.3 g, 6.18 mmol, 3 eq.) and allyl bromide (0.54 ml, 6.18 mmol, 3 eq.) were used following the representative procedure for compound **285**. After work-up and chromatography over silica, the title compound **285f** (518 mg, 89%) was obtained as a white solid.

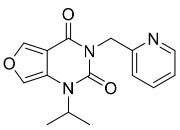


285f

^{1}H NMR (300 MHz, DMSO-d6): δ = 4.37 (d, J = 4.5 Hz, 2H), 5.03 (s, 2H), 5.14-5.31 (m, 2H), 5.75-5.91 (m, 1H), 7.17-7.36 (m, 5H), 7.91 (d, J = 1.7 Hz, 1H), 8.56 (d, J = 1.7 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d6): δ = 44.2, 48.2, 110.95, 118.4, 127.0, 127.6, 127.8, 127.9, 128.9, 131.7, 138.05, 145.9, 150.7, 158.5. IR: 1710, 1665, 1638, 1390, 1342, 1305, 1263, 758, 708 cm^{-1}. MS (ESI, 70 eV): m/z (%) = 283 (100) $[M+H]^+$. HRMS: m/z $[M + H]^+$ calcd for $C_{16}H_{14}N_2O_3$: 283.1083; found: 283.1076. Mp 101-102 °C; R_f = 0.20 (hexanes-EtOAc, 5:1).

1-Isopropyl-3-(pyridin-2-ylmethyl)furo[3,4-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (285g)

38c (500 mg, 2.06 mmol, 1 eq.) in dry DMF (20 mL), Cs_2CO_3 (2.0 g, 6.18 mmol, 3 eq.), TBAI (2.3 g, 6.18 mmol, 3 eq.) and 2-bromopropane (0.58 ml, 6.18 mmol, 3 eq.) were used following the representative procedure for compound **285**. After work-up, chromatography over silica and subsequent recrystallization from EtOH, the title compound **285g** (159 mg, 35%) was obtained as off-white crystals.

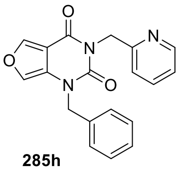


285g

^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.45 (d, J = 7.1 Hz, 6H), 4.93 (septet, J = 7.1 Hz, 1H), 5.31 (s, 2H), 7.13 (dd, J = 7.5 Hz, J = 5.0 Hz, 1H), 7.22 (d, J = 7.5 Hz, 1H), 7.43 (d, J = 1.7 Hz, 1H), 7.62 (dt, J = 7.5 Hz, J = 1.9 Hz, 1H), 8.11 (d, J = 1.7 Hz, 1H), 8.52 (dd, J = 5.0 Hz, J = 1.9 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 18.9, 45.9, 48.8, 111.7, 121.25, 122.1, 125.1, 125.45, 136.5, 144.2, 149.6, 150.7, 156.55, 158.4. IR: 1711, 1670, 1631, 1265, 760, 751 cm^{-1}. MS (ESI, 70 eV): m/z (%) = 286 (100) $[M+H]^+$. HRMS: m/z $[M + H]^+$ calcd for $C_{15}H_{15}N_3O_3$: 286.1192; found: 286.1191. Mp 120-121 °C; R_f = 0.14 (hexanes-EtOAc, 1:1).

1-Benzyl-3-(pyridin-2-ylmethyl)furo[3,4-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (285h)

38c (500 mg, 2.06 mmol, 1 eq.) in dry DMF (20 mL), Cs_2CO_3 (2.0 g, 6.18 mmol, 3 eq.), TBAI (2.3 g, 6.18 mmol, 3 eq.) and benzyl bromide (0.74 ml, 6.18 mmol, 3 eq.) were used following the representative procedure for compound **285**. After work-up, chromatography over silica and subsequent recrystallization from EtOH, the title compound **285h** (570 mg, 83%) was obtained as off-white crystals.

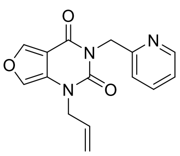


285h

^{1}H NMR (300 MHz, $CDCl_3$): δ = 5.02 (s, 2H), 5.37 (s, 2H), 7.15 (dd, J = 7.7 Hz, J = 5.0 Hz, 1H), 7.19 (d, J = 1.7 Hz, 1H), 7.26 (d, J = 7.7 Hz, 1H), 7.29-7.39 (m, 5H), 7.63 (dt, J = 7.7 Hz, J = 1.7 Hz, 1H), 8.05 (d, J = 1.7 Hz, 1H), 8.53 (dd, J = 5.0 Hz, J = 1.7 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 45.9, 49.5, 111.1, 121.4, 122.2, 125.3, 127.6, 127.9, 128.15, 129.0, 135.15, 136.6, 144.5, 149.6, 151.5, 156.3, 158.5. IR: 1719, 1672, 1639, 758, 695 cm^{-1}. MS (ESI, 70 eV): m/z (%) = 334 (100) $[M+H]^+$. HRMS: m/z $[M + H]^+$ calcd for $C_{19}H_{15}N_3O_3$: 334.1192; found: 334.1190. Mp 125-126 °C; R_f = 0.18 (hexanes-EtOAc, 1:1).

1-Allyl-3-(pyridin-2-ylmethyl)furo[3,4-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (285i)

38c (500 mg, 2.06 mmol, 1 eq.) in dry DMF (20 mL), Cs_2CO_3 (2.0 g, 6.18 mmol, 3 eq.), TBAI (2.3 g, 6.18 mmol, 3 eq.) and allyl bromide (0.54 ml, 6.18 mmol, 3 eq.) were used following the representative procedure for compound **285**. After work-up, chromatography over silica and subsequent recrystallization from EtOH, the title compound **285i** (402 mg, 69%) was obtained as off-white crystals.

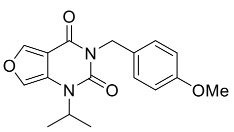


285i

^{1}H NMR (300 MHz, DMSO-d6): δ = 4.37 (d, J = 4.5 Hz, 2H), 5.15 (s, 2H), 5.15-5.33 (m, 2H), 5.74-5.90 (m, 1H), 7.18-7.31 (m, 2H), 7.72 (t, J = 7.8 Hz, 1H), 7.94 (d, J = 1.1 Hz, 1H), 8.42 (d, J = 4.8 Hz, 1H), 8.58 (d, J = 1.1 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d6): δ = 45.5, 48.1, 111.0, 118.3, 121.2, 122.7, 127.0, 127.9, 131.7, 137.2, 145.9, 149.4, 150.7, 156.65, 158.5. IR: 1704, 1663, 1639, 1323, 759, 749 cm^{-1}. MS (ESI, 70 eV): m/z (%) = 284 (100) $[M+H]^+$. HRMS: m/z $[M + H]^+$ calcd for $C_{15}H_{13}N_3O_3$: 284.1035; found: 284.1030. Mp 113-114 °C; R_f = 0.25 (hexanes-EtOAc, 1:1).

1-Isopropyl-3-(4-methoxybenzyl)furo[3,4-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (285j)

38d (500 mg, 1.84 mmol, 1 eq.) in dry DMF (20 mL), Cs_2CO_3 (1.8 g, 5.52 mmol, 3 eq.), TBAI (2.0 g, 5.52 mmol, 3 eq.) and 2-bromopropane (0.52 ml, 5.52 mmol, 3 eq.) were used following the representative procedure for compound **285**. In this case, the reaction mixture was stirred at room temperature for 48 hours. After work-up, chromatography over silica and subsequent recrystallization from EtOH, the title compound **285j** (276 mg, 48%) was obtained as white crystals.

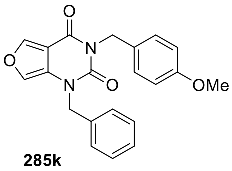


285j

^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.42 (d, J = 6.8 Hz, 6H), 3.77 (s, 3H), 4.94 (septet, J = 6.8 Hz, 1H), 5.10 (s, 2H), 6.83 (d, J = 8.6 Hz, 2H), 7.37 (s, 1H), 7.46 (d, J = 8.6 Hz, 2H), 8.07 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 18.85, 43.9, 48.7, 55.3, 111.8, 113.8, 124.95, 125.2, 129.7, 130.7, 144.0, 150.7, 158.4, 159.1. IR (ATR): 1703, 1656, 1631, 1265, 1241 cm^{-1}. MS (ESI, 70 eV): m/z (%) = 315 (100) $[M+H]^+$. HRMS: m/z $[M + H]^+$ calcd for $C_{17}H_{18}N_2O_4$: 315.1345; found: 315.1341. Mp 109-110 °C; R_f = 0.26 (hexanes-EtOAc, 5:1).

1-Benzyl-3-(4-methoxybenzyl)furo[3,4-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (285k)

38d (500 mg, 1.84 mmol, 1 eq.) in dry DMF (20 mL), Cs_2CO_3 (1.8 g, 5.52 mmol, 3 eq.), TBAI (2.0 g, 5.52 mmol, 3 eq.) and benzyl bromide (0.66 ml, 5.52 mmol, 3 eq.) were used following the representative procedure for compound **285**. After work-up, chromatography over silica and subsequent recrystallization from EtOH, the title compound **285k** (464 mg, 70%) was obtained as a white solid.

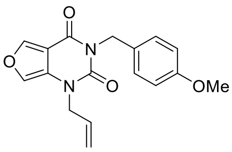


285k

^{1}H NMR (300 MHz, $CDCl_3$): δ = 3.78 (s, 3H), 4.98 (s, 2H), 5.15 (s, 2H), 6.84 (d, J = 7.2 Hz, 2H), 7.14 (s, 1H), 7.24-7.40 (m, 5H), 7.47 (d, J = 7.2 Hz, 2H), 8.02 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 44.1, 49.6, 55.3, 111.2, 113.85, 125.2, 127.6, 127.8, 128.2, 129.0, 129.6, 130.7, 135.15, 144.3, 151.4, 158.4, 159.15. IR: 1706, 1668, 1645, 1297, 1248, 1022, 738, 694 cm^{-1}. MS (ESI, 70 eV): m/z (%) = 363 (100) $[M+H]^+$. HRMS: m/z $[M + H]^+$ calcd for $C_{21}H_{18}N_2O_4$: 363.1345; found: 363.1343. Mp 186-187 °C; R_f = 0.23 (hexanes-EtOAc, 5:1).

1-Allyl-3-(4-methoxybenzyl)furo[3,4-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (285l)

38d (500 mg, 1.84 mmol, 1 eq.) in dry DMF (20 mL), Cs_2CO_3 (1.8 g, 5.52 mmol, 3 eq.), TBAI (2.0 g, 5.52 mmol, 3 eq.) and allyl bromide (0.48 ml, 5.52 mmol, 3 eq.) were used following the representative procedure for compound **285**. After work-up, chromatography over silica and subsequent recrystallization from EtOH, the title compound **285l** (485 mg, 84%) was obtained as white crystals.

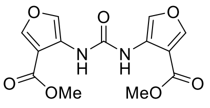


285l

^{1}H NMR (300 MHz, DMSO-d6): δ = 3.69 (s, 3H), 4.36 (d, J = 4.8 Hz, 2H), 4.96 (s, 2H), 5.12-5.32 (m, 2H), 5.73-5.91 (m, 1H), 6.84 (d, J = 8.3 Hz, 2H), 7.23 (d, J = 8.3 Hz, 2H), 7.89 (s, 1H), 8.54 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 43.6, 48.2, 55.6, 111.0, 114.25, 118.5, 126.9, 127.7, 129.8, 130.1, 131.7, 145.85, 150.7, 158.4, 159.0. IR: 1703, 1665, 1636, 1298, 1246, 1031, 933 cm^{-1}. MS (ESI, 70 eV): m/z (%) = 313 (100) $[M+H]^+$. HRMS: m/z $[M + H]^+$ calcd for $C_{17}H_{16}N_2O_4$: 313.1188; found: 313.1181. Mp 106-107 °C; R_f = 0.23 (hexanes-EtOAc, 5:1).

Methyl 4-[3-(4-methoxycarbonylfuran-3-yl)ureido]furan-3-carboxylate (286)

DPPA (4.7 mL, 1.05 eq.) in dry toluene (100 mL) and a solution of 4-(methoxycarbonyl)furan-3-carboxylic acid **283** (3.5 g, 20.6 mmol, 1 eq.) and dry Et_3N (4.1 mL, 1.4 eq.) in dry toluene (50 mL) and were used following the representative procedure for compound **36**. After stirring at 75 °C for three hours, the reaction is cooled down to room temperature, and 0.19 mL of water (0.5 eq.) is added dropwise to the reaction After stirring for ten minutes at room temperature, 0.03 mL of water (0.08 eq.) is added to the reaction, and stirring continued for twelve hours at room temperature. After evaporation of the solvent, the residue was taken up in EtOAc (200 mL) and was added in a separation funnel together with water (200 mL). While frequently shaking, the aqueous layer was neutralized with dilute HCl (3N). After separation, the aqueous layer was extracted again with EtOAc (100 mL). The combined organic extracts were washed with brine (100 mL), dried over magnesium sulfate and evaporated *in vacuo*. The product is triturated in 10 mL of methanol, filtered off and dried to afford 2.137 g of a white solid (Y = 67%).

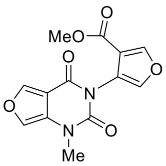


286

^{1}H NMR (400 MHz, DMSO-d$_6$): δ = 3.83 (s, 6H, OC$\underline{H}_3$), 8.09 (d, *J* = 1.7 Hz, 2H, OC$\underline{H}$C$_q$N), 8.28 (d, *J* = 1.7 Hz, 2H, OC$\underline{H}$C$_q$C(O)), 9.35 (s, 2H, N$\underline{H}$). ^{13}C NMR (100 MHz, DMSO-d$_6$): δ = 51.5 (O$\underline{C}$H$_3$), 110.5 ($\underline{C}_q$C(O)OMe), 124.6 ($\underline{C}_q$NHC(O)), 132.1 (O$\underline{C}$HC$_q$NH), 147.4 (O$\underline{C}$HC$_q$C(O)OMe), 152.1 (NH$\underline{C}$(O)NH), 163.0 ($\underline{C}$(O)OMe). IR: 1703, 1668, 1547, 1526, 1314, 1258, 1152, 1103, 1030, 770, 571, 548 cm^{-1}. MS (ESI, 70 eV): m/z (%) = 309.1 (100, [M + H]$^+$). Mp 214-215 °C

3-(4-Methoxycarbonylfuran-3-yl)furo[3,4-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (288)

132 mg of compound **286** (0.46 mmol; 1 eq.) is dissolved in 5 mL of dry DMF under an atmosphere of nitrogen. Then, 837 mg of cesium carbonate (6 eq.) and 949 mg of TBAI (6 eq.) are added. After stirring for thirty minutes at room temperature, 0.16 mL of iodomethane (6 eq.) is added. Stirring continued for eighteen hours at room temperature. The reaction mixture is poured in 50 mL of water and extracted with ethyl acetate (3 x 50 mL). The combined organic layers are washed with brine (50 mL), dried over magnesium sulfate and the solvent is removed *in vacuo*. The crude product is triturated in 1 mL of ethyl acetate. After removal of the ethyl acetate by decantation, 51 mg of an off-white solid is obtained (Y = 41%).

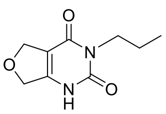


288

^{1}H NMR (400 MHz, DMSO-d$_6$): δ = 3.31 (s, 3H), 3.66 (s, 3H), 8.01 (d, *J* = 1.6 Hz, 1H), 8.04 (d, *J* = 1.5 Hz, 1H), 8.51 (d, *J* = 1.6 Hz, 1H), 8.64 (d, *J* = 1.5 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d$_6$): δ = 32.3, 51.5, 110.2, 115.8, 120.9, 126.7, 128.5, 143.2, 146.1, 149.0, 149.9, 157.5, 161.5. IR: 1682, 1645, 1377, 1167, 758, 596 cm^{-1}. MS (ESI, 70 eV): m/z (%) = 291.1 (100, [M + H]$^+$). Mp 191-192 °C.

3-Propyl-5,7-dihydrofuro[3,4-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (298)

MeOH (2 mL) was added to a mixture of **38a** (75 mg, 0.39 mmol, 1 eq.) and Pd/C (10 wt. % Pd; 21 mg, 5 mol%). The reaction mixture was stirred at room temperature under a H_2 atmosphere (5 bar) for 1 h, filtered over a pad of celite (1 cm) and rinsed with MeOH (2x 10 mL). The solvent was evaporated *in vacuo*, and the crude product was purified by column chromatography over silica and subsequently recrystallized from MeOH to give the title compound **298** (54 mg, 71%) as white crystals.

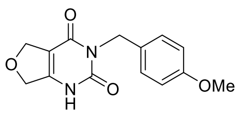


298

^{1}H NMR (400 MHz, CDCl$_3$): δ = 0.96 (t, *J* = 7.5 Hz, 3H, C$\underline{H}_3$), 1.66 (sextet, *J* = 7.5 Hz, 2H, C$\underline{H}_2$CH$_3$), 3.89 (t, *J* = 7.5 Hz, 2H, NC$\underline{H}_2$), 4.90 (t, *J* = 3.6 Hz, 2H, OC$\underline{H}_2$C$_q$C(O)), 4.96 (t, *J* = 3.6 Hz, 2H, OC$\underline{H}_2$C$_q$NH), 10.88 (br. s, 1H, N$\underline{H}$). ^{13}C NMR (100 MHz, CDCl$_3$): δ = 11.3 ($\underline{C}$H$_3$), 21.0 ($\underline{C}$H$_2$CH$_3$), 42.3 (N$\underline{C}$H$_2$), 71.0 (O$\underline{C}$H$_2$C$_q$C(O)), 72.2 (O$\underline{C}$H$_2$C$_q$NH), 108.6 ($\underline{C}_q$C(O)), 149.1 ($\underline{C}_q$NHC(O)), 154.0 (NH$\underline{C}$(O)N, 158.8 (C$_q\underline{C}$(O)N). IR: 1713, 1670, 1622, 1538, 1444, 1418, 1334, 1060, 760, 736 cm^{-1}. MS (ESI, 70 eV): m/z (%) = 195 (100) [M - H]$^-$. HRMS: m/z [M - H]$^-$ calcd for $C_9H_{12}N_2O_3$: 195.0775; found: 195.0776. Mp 179-180 °C; R$_f$ = 0.20 (hexanes-EtOAc, 1:1).

3-(4-Methoxybenzyl)-5,7-dihydrofuro[3,4-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (300)

MeOH (1 mL) was added to a mixture of **38d** (50 mg, 0.18 mmol, 1 eq.) and Pd/C (10 wt. % Pd; 10 mg, 5 mol%). The reaction mixture was stirred at room temperature under a H_2 atmosphere (5 bar) for 1 h, filtered over a pad of celite (1 cm) and rinsed with MeOH (2x 10 mL). The solvent was evaporated *in vacuo*, and the crude product was purified by column chromatography over silica and subsequently recrystallized from MeOH to give the title compound **300** (33 mg, 66%) as white crystals

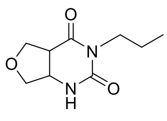


300

^{1}H NMR (400 MHz, $CDCl_3$): δ = 3.77 (s, 3H), 4.85 (t, *J* = 3.6 Hz, 2H), 4.95 (t, *J* = 3.6 Hz, 2H), 5.02 (s, 2H), 6.82 (d, *J* = 8.7 Hz, 2H), 7.39 (d, *J* = 8.7 Hz, 2H), 10.75 (br. s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 43.3, 55.3, 71.0, 72.2, 108.7, 113.8, 128.7, 130.6, 149.2, 154.0, 158.6, 159.2. IR: 1709, 1633, 1514, 1246, 1030, 666 cm^{-1}. MS (ESI, 70 eV): m/z (%) = 273 (100) $[M - H]^-$. HRMS: m/z $[M - H]^-$ calcd for $C_{14}H_{14}N_2O_4$: 273.0881; found: 273.0892. Mp 227-228 °C; R_f = 0.19 (hexanes-EtOAc, 1:1).

3-Propyltetrahydrofuro[3,4-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (299)

MeOH (40 mL) was added to a mixture of **38a** (815 mg, 4.2 mmol, 1 eq.) and Rh/Al (5 wt. % Rh; 432 mg, 5 mol%). The reaction mixture was stirred at room temperature under a H_2 atmosphere (5 bar) for 12 h, filtered over a pad of celite (1 cm) and rinsed with MeOH (2x 20 mL). The solvent was evaporated *in vacuo*, and the crude product was purified by column chromatography over silica and subsequently recrystallized from MeOH to give the title compound **299** (662 mg, 79%) as white crystals.

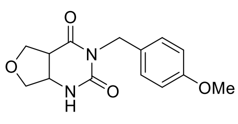


299

^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.91 (t, *J* = 7.4 Hz, 3H, $C\underline{H}_3$), 1.58 (sextet, *J* = 7.4 Hz, 2H, $C\underline{H}_2CH_3$), 3.24-3.32 (m, 1H, $C\underline{H}C(O)$), 3.70-3.79 (m, 2H, $NC\underline{H}_2$), 3.84 (dd, *J* = 9.5 Hz, *J* = 3.1 Hz, 1H, $OC\underline{H}_aH_bCHNH$), 3.97 (dd, *J* = 9.5 Hz, *J* = 4.7 Hz, 1H, $OCH_a\underline{H}_bCHNH$), 4.08-4.17 (m, 2H, $OC\underline{H}_aH_bCHC(O)$, $C\underline{H}NH$), 4.24 (t, *J* = 8.8 Hz, 1H, $OCH_a\underline{H}_bCHC(O)$), 5.96 (br. s, 1H, $N\underline{H}$). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 11.2 ($\underline{C}H_3$), 21.6 ($\underline{C}H_2CH_3$), 42.0 ($N\underline{C}H_2$), 43.6 ($\underline{C}HC(O)$), 50.7 ($\underline{C}HNH$), 70.9 ($O\underline{C}H_2CHC(O)$), 75.0 ($O\underline{C}H_2CHNH$), 153.3 ($NH\underline{C}(O)N$), 168.8 ($CH\underline{C}(O)N$)). IR : 1714, 1671, 1449, 1364, 1246, 1045, 765, 720 cm^{-1}. MS (ESI, 70 eV): m/z (%) = 197 (100) $[M - H]^-$. HRMS: m/z $[M - H]^-$ calcd for $C_9H_{14}N_2O_3$: 197.0932; found: 197.0934. Mp 110-111 °C; R_f = 0.28 (EtOAc).

3-(4-Methoxybenzyl)tetrahydrofuro[3,4-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (301)

MeOH (2 mL) was added to a mixture of **38d** (50 mg, 0.18 mmol, 1 eq.) and Rh/Al (5 wt. % Rh; 19 mg, 5 mol%). The reaction mixture was stirred at room temperature under a H_2 atmosphere (5 bar) for 12 h, filtered over a pad of celite (1 cm) and rinsed with MeOH (2x 20 mL). The solvent was evaporated *in vacuo*, and the crude product was purified by column chromatography over silica and subsequently recrystallized from MeOH to give the title compound **301** (36 mg, 71%) as white crystals.



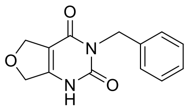
301

^{1}H NMR (400 MHz, $CDCl_3$): δ = 3.24-3.32 (m, 1H), 3.78 (s, 3H), 3.72-3.84 (m, 1H), 3.94 (dd, *J* = 9.5 Hz, *J* = 4.8 Hz, 1H), 4.05-4.13 (m, 2H), 4.18-4.25 (m, 1H), 4.90 (s, 2H), 6.19 (br. s, 1H), 6.82 (d, *J* = 8.7 Hz, 2H), 7.33 (d, *J* = 8.7 Hz, 2H).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 43.0, 43.6, 50.7, 55.2, 70.7, 74.9, 113.8, 129.5, 130.2, 153.0, 159.0, 168.6. IR: 1716, 1667, 1514, 1455, 1249, 1175, 1033, 768, 719 cm^{-1}. MS (ESI, 70 eV): m/z (%) = 277 (100) $[M + H]^+$. HRMS: m/z $[M + H]^+$ calcd for $C_{14}H_{16}N_2O_4$: 277.1183; found: 277.1171. Mp 141-142 °C; R_f = 0.14 (hexanes-EtOAc, 1:1).

3-Benzyl-5,7-dihydrofuro[3,4-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (302)

MeOH (5 mL) was added to a mixture of **38b** (100 mg, 0.41 mmol, 1 eq.) and Pd/C (10 wt. % Pd; 20 mg, 5 mol%). The reaction mixture was stirred at room temperature under a H_2 atmosphere (5 bar) for 24 h, filtered over a pad of celite (1 cm) and rinsed with MeOH (2x 20 mL). The solvent was evaporated *in vacuo*, and the crude product was recrystallized from ethanol and subsequently recrystallized from acetonitrile to afford 37 mg of white crystals (Y = 36%).

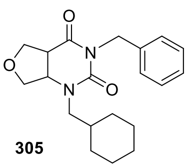


302

^1H NMR (400 MHz, DMSO-d_6): δ = 4.78 (s, 4H), 4.95 (s, 2H), 7.18-7.35 (m, 5H), 11.71 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ = 42.7, 70.5, 71.0, 105.7, 127.1, 127.5, 128.3, 137.3, 151.1, 151.8, 158.7. IR: 1665, 1628, 1605, 1414, 698, 669 cm^{-1}. MS (ESI, 70 eV): m/z (%) = 245.2 (100, $[M + H]^+$). Mp 239-240 °C.

3-Benzyl-1-(cyclohexylmethyl)tetrahydrofuro[3,4-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (305)

MeOH (5 mL) was added to a mixture of **285e** (100 mg, 0.3 mmol, 1 eq.) and Rh/Al (5 wt. % Rh; 31 mg, 5 mol%). The reaction mixture was stirred at room temperature under a H_2 atmosphere (5 bar) for 60 h, filtered over a pad of celite (1 cm) and rinsed with MeOH (2x 20 mL). The solvent was evaporated *in vacuo*, and the crude product was purified by preparative TLC (AcOEt/hexane (1:3); R_f = 0.2) and subsequently preparative HPLC (water/acetonitrile; gradient from 60% to 65% acetonitrile over 6 column volumes) to obtain 21 mg of a colourless oil (Y = 20%). The position of the hexyl ring and the benzene ring was elucidated by a combination of ^1H-^1H COSY, HSQC and HMBC NMR techniques.

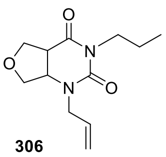


305

^1H NMR (400 MHz, $CDCl_3$): δ = 0.85-1.08 (m, 2H), 1.10-1.30 (m, 3H), 1.57-1.80 (m, 6H), 2.96 (dd, *J* = 7.1 Hz, *J* = 13.9 Hz, 1H), 3.23 (ddd, *J* = 3.0 Hz, *J* = 7.1 Hz, *J* = 8.4 Hz, 1H), 3.47 (t, *J* = 8.4 Hz, 1H), 3.60 (dd, *J* = 7.1 Hz, *J* = 13.9 Hz, 1H), 3.93-4.01 (m, 1H), 4.06 (dd, *J* = 6.9 Hz, *J* = 8.4 Hz, 1H), 4.12 (dd, *J* = 7.1 Hz, *J* = 9.1 Hz, 1H), 4.40 (dd, *J* = 3.0 Hz, *J* = 9.1 Hz, 1H), 4.98 (d, *J* = 14.0 Hz, 1H), 5.05 (d, *J* = 14.0 Hz, 1H), 7.19-7.39 (m, 5H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 25.7, 25.8, 26.4, 30.7, 31.0, 36.7, 42.9, 44.5, 54.6, 56.0, 70.8, 71.5, 127.4, 128.5, 128.6, 137.7, 151.8, 169.0. IR: 2922, 2850, 1705, 1664, 1464, 1450, 1213, 699 cm^{-1}. MS (ESI, 70 eV): m/z (%) = 343.0 (100, $[M + H]^+$). HRMS: m/z $[M + H]^+$ calcd for $C_{20}H_{27}N_2O_3$: 343.2016; found: 343.2015.

1-Allyl-3-propyltetrahydrofuro[3,4-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (306)

To a solution of **299** (200 mg, 1 mmol, 1 eq.) in dry DMF (10 mL), Cs_2CO_3 (986 mg, 3 eq.) and TBAI (1.12 g, 3 eq.) were added under an inert atmosphere. After stirring the reaction for 30 minutes at room temperature, allyl bromide (0.26 ml, 3 eq.) was added and stirring continued at room temperature for 12 hours. Water (50 mL) was added, and the reaction mixture was extracted with EtOAc (3x 50 mL). The combined organic layers were combined, washed with brine (50 mL), dried over $MgSO_4$ and evaporated *in vacuo.* The crude product was purified by column chromatography over silica (AcOEt/hexane (1:3)) to afford 144 mg of a yellow oil (Y = 60%).

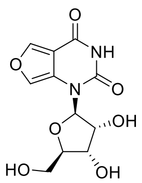


306

^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.84 (t, *J* = 7.5 Hz, 3H), 1.52 (sextet, *J* = 7.5 Hz, 2H), 3.17 (dt, *J* = 3.5 Hz, *J* = 12.1 Hz, 1H), 3.45-3.52 (m, 1H), 3.65-3.80 (m, 2H), 3.87-4.03 (m, 3H), 4.06 (dd, *J* = 7.3 Hz, *J* = 9.1 Hz, 1H), 4.12 (ddt, *J* = 1.3 Hz, *J* = 6.0 Hz, *J* = 15.3 Hz, 1H), 4.27 (dd, *J* = 3.5 Hz, *J* = 9.1 Hz, 1H), 5.16-5.25 (m, 2H), 5.70-5.83 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 11.2, 21.5, 42.8, 42.8, 50.6, 54.7, 70.8, 71.8, 118.7, 132.6, 151.3, 168.8. IR: 2962, 1703, 1660, 1461, 1220, 1070, 928, 753 cm^{-1}. MS (ESI, 70 eV): m/z (%) = 239.1 (100, $[M + H]^+$). HRMS: m/z $[M + H]^+$ calcd for $C_{12}H_{19}N_2O_3$: 239.1390; found: 239.1397.

1-((2*R*,3*R*,4*S*,5*R*)-3,4-Dihydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)furo[3,4-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (314)

To a suspension of **6** (110 mg; 0.72 mmol; 1 eq.) in 7 mL of dry acetonitrile, 0.53 mL of *N*,*O*-bis(trimethylsilyl)acetamide (3 eq.). was added dropwise The mixture was stirred for two hours at room temperature under an inert atmosphere. To the reaction mixture were successively added a solution of 343 mg of ATBR (0.94 eq.) in 3 mL of acetonitrile, and 0.21 mL of TMSOTf (1.6 eq.). The reaction was heated at 85 °C for 45 minutes, cooled down to room temperature and the solvent was removed *in vacuo*. The residue was dissolved in 10 mL of dichloromethane, washed with 10 mL of sat. aqueous sodium bicarbonate and 10 mL of brine, dried over magnesium sulfate and the solvent was removed *in vacuo*. The crude product was purified by column chromatography over silica (AcOEt/hexane, gradient from 0% to 100% ethyl acetate over 15 column volumes; R_f = 0.64 (AcOEt/hexane (1:1))). The obtained product (300 mg) was dissolved in 10 mL of 7M ammonia in methanol solution and stirred at room temperature for 12 hours. The solvent was removed *in vacuo*, and the crude product was purified by column chromatography over silica (dichloromethane/methanol, gradient from 0% to 10% methanol over 15 column volumes; R_f = 0.19 (10% methanol in dichloromethane). Compound **314** was obtained as a white solid (114 mg, Y = 55%).



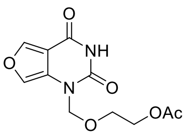
314

^{1}H NMR (400 MHz, DMSO-d_6): δ = 3.58-3.69 (m, 2H, C$\underline{H}_2$OH), 3.80-3.85 (m, 1H, 4'-$\underline{H}$), 4.00-4.07 (m, 1H, 3'-$\underline{H}$), 4.27 (dd, *J* = 6.8 Hz, *J* = 13.3 Hz, 1H, 2'-$\underline{H}$), 5.04 (d, *J* = 4.8 Hz, 1H, 3'-O$\underline{H}$), 5.17-5.24 (m, 2H, 2'-O$\underline{H}$, 5'-O$\underline{H}$), 6.00 (d, *J* = 6.8 Hz, 1H, 1'-$\underline{H}$), 8.17 (d, *J* = 1.6 Hz, 1H, C$\underline{H}$C$_q$N), 8.49 (d, *J* = 1.6 Hz, 1H, C$\underline{H}$C$_q$C(O)), 11.16 (br. s, 1H, N$\underline{H}$). ^{13}C NMR (100 MHz, DMSO-d_6): δ = 61.4 ($\underline{C}$H$_2$OH), 68.6 (2'-$\underline{C}$), 69.7 (3'-$\underline{C}$), 85.5 (4'-$\underline{C}$), 87.6 (1'-$\underline{C}$), 111.9 (CH$\underline{C}_q$C(O)), 124.8 (CH$\underline{C}_q$N), 128.9 ($\underline{C}$HC$_q$N), 144.5 ($\underline{C}$HC$_q$C(O)), 151.0 (N$\underline{C}$(O)NH), 158.6 (Cq$\underline{C}$(O)NH). IR: 3402, 3186, 3102, 1698, 1682, 1638, 1297, 1080, 1041, 1028 cm^{-1}. MS (ESI, 70 eV): m/z (%) = 283.0 (100, $[M - H]^-$). HRMS: m/z $[M - H]^-$ calcd for $C_{11}H_{11}N_2O_7$: 283.0572; found: 283.0572. Mp 170-171 °C.

1-((2-Acetoxyethoxy)methyl)furo[3,4-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (315)

The synthesis of 2-(chloromethoxy)ethyl acetate was accomplished following the procedure of Manvar and Shah.[209] All spectra were in accordance with the reported spectral data.

To a stirred suspension of **6** (300 mg, 1.97 mmol, 1 eq.) in 24 mL of dry dichloromethane, 1.06 mL of *N*,*O*-BSA (2.2 eq.) was added dropwise at room temperature under an inert atmosphere. After stirring for an additional 30 minutes, 7.3 mg of TBAI (0.01 eq.) was added, the reaction mixture was cooled down to 0 °C and 451 mg of 2-(chloromethoxy)ethyl acetate (1.5 eq.) was added dropwise. Stirring was continued for 2 hours at 0 °C. The mixture was poured into ice cold sat. aqueous sodium bicarbonate (60 mL) and stirred at 0 °C for 30 minutes. The organic layer was separated and the aqueous layer was extracted with dichloromethane (2 x 60 mL). The combined organic layers were washed with brine (2 x 90 mL), dried over magnesium sulfate and the solvent was removed *in vacuo*. The crude product was purified by column chromatography over silica (dichloromethane/methanol, gradient from 0% methanol to 5% methanol over 15 column volumes; R_f = 0.4 (5% methanol in dichloromethane)). Compound **315** was obtained as a white solid (218 mg, Y = 41%).

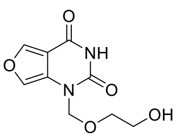


315

^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.03 (s, 3H), 3.77 (t, *J* = 4.6 Hz, 2H), 4.21 (t, *J* = 4.6 Hz, 2H), 5.34 (s, 2H), 7.52 (d, *J* = 1.3 Hz, 1H), 7.95 (br. s, 1H), 8.09 (d, *J* = 1.3 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 20.8, 62.9, 66.9, 74.8, 111.0, 126.8, 127.5, 144.2, 150.4, 157.6, 170.8. IR: 3060, 1728, 1681, 1638, 1291, 1255, 1235, 1128, 1085, 1051, 1033, 712 cm^{-1}. MS (ESI, 70 eV): m/z (%) = 267.0 (100, $[M – H]^-$). HRMS: m/z $[M – H]^-$ calcd for $C_{11}H_{11}N_2O_6$: 267.0623; found: 267.0630. Mp 176-177 °C.

1-((2-Hydroxyethoxy)methyl)furo[3,4-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (316)

A mixture of 56 mg of compound **315** (0.2 mmol) in 5 mL of a 7M ammonia in methanol solution was stirred at room temperature for 12 hours. The solvent was removed in vacuo and the crude product was purified by column chromatography over silica (dichloromethane/methanol, gradient from 0% methanol to 10% methanol over 15 column volumes; R_f = 0.48 (10% methanol in dichloromethane)). Compound **316** is obtained as a white solid (40 mg, Y = 85%).

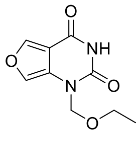


316

^{1}H NMR (400 MHz, MeOH-d_4): δ = 3.59-3.68 (m, 4H, $C\underline{H}_2C\underline{H}_2$), 5.33 (s, 2H, $NC\underline{H}_2O$), 7.74 (d, *J* = 1.6 Hz, 1H, $C\underline{H}C_qN$), 8.24 (d, *J* = 1.6 Hz, 1H, $C\underline{H}C_qC(O)$). ^{13}C NMR (100 MHz, MeOH-d_4): δ = 61.9 ($\underline{C}H_2OH$), 71.5 ($\underline{C}H_2CH_2OH$), 75.8 ($N\underline{C}H_2O$), 112.5 ($\underline{C}_qC(O)$), 128.4 ($\underline{C}HC_qN$), 129.4 ($\underline{C}_qNC(O)$), 145.5 ($\underline{C}HC_qC(O)$), 152.7 ($N\underline{C}(O)NH$), 160.7 ($C_q\underline{C}(O)NH$). IR: 3439, 3030, 2860, 1678, 1634, 1292, 1069, 1015, 883, 723, 584 cm^{-1}. MS (ESI, 70 eV): m/z (%) = 226.7 (7, $[M + H]^+$). HRMS: m/z $[M + H]^+$ calcd for $C_9H_{11}N_2O_5$: 227.0662; found: 227.0671. Mp 197-198 °C.

1-(Ethoxymethyl)furo[3,4-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (317)

To a stirred suspension of **6** (100 mg, 0.66 mmol, 1 eq.) in 8 mL of dry dichloromethane, 0.354 mL of *N*,*O*-BSA (2.2 eq.) was added dropwise at room temperature under an inert atmosphere. After stirring for an additional 30 minutes at room temperature, 2.4 mg of TBAI (0.01 eq.) was added, the reaction mixture was cooled down to 0 °C and 0.09 mL of chloromethyl ethyl ether (1.5 eq.) was added dropwise. Stirring was continued for 2 hours at 0 °C. The mixture was poured into ice cold sat. aqueous sodium bicarbonate (20 mL) and stirred at 0 °C for 30 minutes. The organic layer was separated and the aqueous layer was extracted with dichloromethane (2 x 20 mL). The combined organic layers were washed with brine (2 x 30 mL), dried over magnesium sulfate and the solvent was removed *in vacuo*. The crude product was recrystallized from methanol to obtain compound **317** as white crystals (115 mg, Y = 83%).



317

^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.21 (t, *J* = 7.0 Hz, 3H), 3.61 (q, *J* = 7.0 Hz, 2H), 5.30 (s, 2H), 7.52 (d, *J* = 1.2 Hz, 1H), 8.09 (d, *J* = 1.2 Hz, 1H), 8.01 (br. s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 14.9, 64.6, 74.5, 111.0, 126.8, 127.6, 144.1, 150.5, 158.0. IR: 3083, 1726, 1675, 1637, 1288, 1074, 764, 707 cm^{-1}. MS (ESI, 70 eV): m/z (%) = 209.0 (100, [M – H]$^-$). HRMS: m/z [M – H]$^-$ calcd for $C_9H_9N_2O_4$: 209.0568; found: 209.0574. Mp 179-180 °C

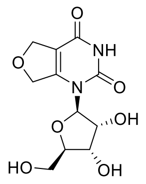
5,7-Dihydrofuro[3,4-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (8)

Compound **8** was synthesized following a literature procedure, and was obtained in 12% yield over two steps. All spectra were in accordance with the reported spectral data.[34]

1-((2*R*,3*R*,4*S*,5*R*)-3,4-Dihydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-5,7-dihydrofuro[3,4-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (319)

To a suspension of 200 mg of **8** (1.3 mmol, 1 eq.) in 14 mL of dry acetonitrile was added dropwise 0.95 mL of *N*,*O*-BSA (3 eq.) and the reaction mixture was stirred for two hours at room temperature under an inert atmosphere. Then, a solution of 616 mg of ATBR (0.94 eq.) in 6 mL of dry acetonitrile was added to the reaction, followed by 0.38 mL of TMSOTf (1.6 eq.). The reaction mixture was stirred at 85 °C for 45 minutes, cooled down to room temperature and the solvent was removed *in vacuo*. The residue was dissolved in 20 mL of dichloromethane and washed with 20 mL of sat. aqueous sodium bicarbonate solution and 20 mL of brine. The organic layer was dried over magnesium sulfate and the solvent was evaporated *in vacuo*. The crude product was purified by column chromatography over silica (dichloromethane/methanol, gradient from 0% methanol to 5% methanol over 10 column volumes, R_f = 0.4 (diethyl ether/dichloromethane (1:3))) and subsequently by preparative HPLC (water/acetonitrile, gradient from 60% acetonitrile to 72.5% acetonitrile over 5 column volumes) to obtain compound **318** (159 mg). This compound was taken up in 20 mL of a 7M ammonia in methanol solution and was stirred at room temperature for two days. The solvent was removed *in vacuo*, and the crude product was purified by column chromatography over silica (ethyl acetate (A)/(ethyl acetate/methanol/water [30:10:5] (B)), gradient from 0% of B to 100% of B over 15 column volumes, R_f = 0.74 (B)) and subsequently recrystallized from absolute ethanol to obtain compound **319** as white crystals (27 mg, Y = 7% over two steps).

^{1}H NMR (400 MHz, MeOH-d$_4$): δ = 3.68 (dd, *J* = 4.9 Hz, *J* = 12.1 Hz, 1H, C$\underline{H}_a$H$_b$OH), 3.78 (dd, *J* = 3.0 Hz, *J* = 12.1 Hz, 1H, CH$_a\underline{H}_b$OH), 3.89-3.95 (m, 1H, 4'-$\underline{H}$), 4.20 (t, *J* = 5.5 Hz, 1H, 3'-$\underline{H}$), 4.53 (t, *J* = 5.5 Hz, 1H, 2'-$\underline{H}$), 4.80-4.90 (m, 2H, OC$\underline{H}_2$C$_q$N), 5.01-5.14 (m, 2H, OC$\underline{H}_2$C$_q$C(O)), 5.35 (d, *J* = 5.5 Hz, 1H,



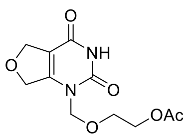
319

1'-H). ^{13}C NMR (100 MHz, MeOH-d_4): δ = 63.2 (CH_2OH), 71.2 (3'-C), 72.2 (OCH_2C_qN), 72.7 ($OCH_2C_qC(O)$), 73.2 (2'-C), 86.9 (4'-C), 94.1 (1'-C), 110.5 ($C_qC(O)$), 153.0 (NC(O)NH), 154.8 (OCH_2C_qN), 161.2($C_qC(O)NH$). IR: 3437, 3146, 3019, 2899, 2803, 1665, 1508, 1128, 1074, 530 cm^{-1}. MS (ESI, 70 eV): m/z (%) = 285 (100, $[M – H]^-$). Mp 177-178 °C.

1-((2-Acetoxyethoxy)methyl)5,7-dihydrofuro[3,4-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (320)

The synthesis of 2-(acetoxyethoxy)methyl acetate was accomplished following the procedure of Boncel *et al*.[140] All spectra were in accordance with the reported spectral data.

To a stirred mixture of compound **8** (72 mg, 0.47 mmol, 1 eq.) in 6 mL of dry dichloromethane , 0.25 mL of *N*,*O*-BSA (2.2 eq.) was added dropwise at room temperature under an inert atmosphere. After stirring at room temperature for three hours, 124 mg of 2-(acetoxyethoxy)methyl acetate (1.5 eq.) was added and the reaction mixture was cooled down to 0 °C. Then, 0.017 mL of TMSOTf (0.2 eq.) was added at 0 °C and the reaction mixture was stirred at room temperature for three days. The reaction was quenched by addition of 10 mL of sat. aqueous sodium bicarbonate and the layers were separated. The aqueous layer was extracted with dichloromethane (4 x 10 mL), the combined organic layers were washed with brine (20 mL), dried over magnesium sulfate and the solvent was removed *in vacuo*. The crude product was purified by column chromatography over silica (dichloromethane/methanol, gradient from 0% to 5% methanol over 15 column volumes, R_f = 0.48 (5% methanol in dichloromethane)) and subsequently purified by preparative HPLC (water/acetonitrile (9:1)). Compound **320** was obtained as a white solid (33 mg, Y = 26%).

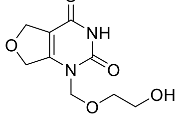


320

^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.08 (s, 3H), 3.76-3.84 (m, 2H), 4.17-4.25 (m, 2H), 4.93-5.00 (m, 2H), 5.01-5.08 (m, 2H), 5.19 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 20.8, 62.9, 67.7, 71.0, 71.9, 74.3, 110.0, 152.1, 153.0, 158.6, 170.8. MS (ESI, 70 eV): m/z (%) = 271.0 (27, $[M + H]^+$). HRMS: m/z $[M + H]^+$ calcd for $C_{11}H_{15}N_2O_6$: 271.0925; found: 271.0916. Mp 101-102 °C

1-((2-Hydroxyethoxy)methyl)-5,7-dihydrofuro[3,4-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (321)

A mixture of compound **320** (18 mg, 0.07 mmol) and 2 mL of a 7M ammonia in methanol solution was stirred at room temperature for 12 hours. The solvent was removed in vacuo, and the crude product was recrystallized from methanol to afford compound **321** as white crystals (14 mg, Y = 92%).

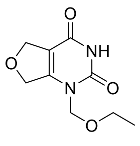


321

^{1}H NMR (400 MHz, MeOH-d_4): δ = 3.59-3.67 (m, 4H, CH_2CH_2), 4.87 (t, J = 3.7 Hz, 2H, OCH_2C_qN), 5.07 (t, J = 3.7 Hz, 2H, $OCH_2C_qC(O)$), 5.19 (s, 2H, NCH_2O). ^{13}C NMR (100 MHz, MeOH-d_4): δ = 61.9 (CH_2OH), 72.1 (CH_2CH_2OH), 72.1 ($OCH_2C_qC(O)$), 72.5 (OCH_2C_qN), 75.6 (NCH_2O), 110.1 ($C_qC(O)$), 154.0 (NC(O)NH), 155.6 ($C_qNC(O)$), 161.4 ($C_qC(O)$). IR: 3422, 3026, 1674, 1638, 1497, 1119, 1070, 1049, 895, 561 cm^{-1}. MS (ESI, 70 eV): m/z (%) = 227.0 (100, $[M – H]^-$). HRMS: m/z $[M – H]^-$ calcd for $C_9H_{11}N_2O_5$: 227.0673; found: 227.0673. Mp 196-197 °C.

1-(Ethoxymethyl)-5,7-dihydrofuro[3,4-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (322)

To a stirred suspension of compound **8** (200 mg, 1.3 mmol, 1 eq.) in 16 mL of dry dichloromethane, 0.7 mL of *N*,*O*-BSA (2.2 eq.) was added dropwise at room temperature under an inert atmosphere. After stirring for an additional 30 minutes, 4.8 mg of TBAI (0.01 eq.) was added, the reaction mixture was cooled down to 0 °C and 0.18 mL of chloromethyl ethyl ether (1.5 eq.) was added dropwise. Stirring was continued for two hours at 0 °C. The mixture was poured into ice cold sat. aqueous sodium bicarbonate (40 mL) and stirred at 0 °C for 30 minutes. The organic layer was separated and the aqueous layer was extracted with dichloromethane (2 x 20 mL). The combined organic layers were washed with brine (2 x 30 mL), dried over magnesium sulfate and the solvent was removed *in vacuo*. The crude product was recrystallized from methanol to obtain compound **322** as white crystals (135 mg, Y = 49%).

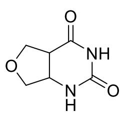


322

^{1}H NMR (400 MHz, DMSO-d$_6$): δ = 1.10 (t, *J* = 7.0 Hz, 3H), 3.50 (q, *J* = 7.0 Hz, 2H), 4.77 (t, *J* = 3.7 Hz, 2H), 4.96 (t, *J* = 3.7 Hz, 2H), 5.06 (s, 2H), 11.38 (br. s, 1H). ^{13}C NMR (100 MHz, DMSO-d$_6$): δ = 14.8, 63.9, 70.5, 71.0, 73.5, 108.1, 152.0, 153.0, 158.8. IR: 3048, 1686, 1649, 1491, 1051 cm^{-1}. MS (ESI, 70 eV): m/z (%) = 211.1 (100, [M – H]$^-$). HRMS: m/z [M – H]$^-$ calcd for $C_9H_{11}N_2O_4$: 211.0724; found: 211.0731. Mp 149-150 °C.

Tetrahydrofuro[3,4-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (323)

A suspension of 500 mg of **6** (3.3 mmol; 1 eq.) and 338 mg of 5 wt% rhodium on alumina (5 mol%) in 50 mL of a methanol/water mixture (4:1) was stirred for 28 hours at room temperature under a hydrogen atmosphere of 5 bar. The reaction mixture was filtered over a pad of celite and rinsed with hot water (40~50 °C, 2 x 20 mL). The solvent was evaporated *in vacuo*, and the residue was recrystallized from methanol to obtain compound **323** (mixture of cis-isomers) as off-white crystals (267 mg, Y = 52%).

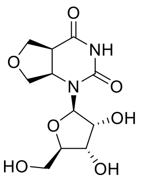
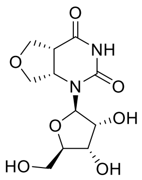


323

^{1}H NMR (400 MHz, DMSO-d$_6$): δ = 3.12-3.20 (m, 1H, $C\underline{H}C(O)$), 3.63 (dd, *J* = 3.5 Hz, *J* = 9.0 Hz, 1H, $OC\underline{H}_aH_bCHNH$), 3.76 (dd, *J* = 5.1 Hz, *J* = 9.0 Hz, 1H, $OCH_a\underline{H}_bCHNH$), 3.88 (dd, *J* = 6.2 Hz, *J* = 8.5 Hz, 1H, $OC\underline{H}_aH_bCHC(O)$), 4.00 (t, *J* = 8.5 Hz, 1H, $OCH_a\underline{H}_bCHC(O)$), 4.02-4.08 (m, 1H, $C\underline{H}NH$), 7.63 (s, 1H, $CHN\underline{H}$), 10.23 (s, 1H, $C(O)N\underline{H}C(O)$). ^{13}C NMR (100 MHz, DMSO-d$_6$): δ = 42.1 ($\underline{C}HC(O)$), 51.2 ($\underline{C}HNH$), 69.7 ($O\underline{C}H_2CHC(O)$), 73.9 ($O\underline{C}H_2CHNH$), 152.1 ($NH\underline{C}(O)NH$), 170.5 ($CH\underline{C}(O)$). IR: 3188, 3090, 1680, 1261, 760, 557, 500, 449 cm^{-1}. MS (ESI, 70 eV): m/z (%) = 155.1 (100, [M – H]$^-$). HRMS: m/z [M – H]$^-$ calcd for $C_6H_7N_2O_3$: 155.0462; found: 155.0460. Mp 213-214 °C.

1-((2*R*,3*R*,4*S*,5*R*)-3,4-Dihydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)tetrahydrofuro[3,4-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (325)

A suspension of 61 mg of **314** (0.215 mmol, 1 eq.) and 22 mg of 5 wt% rhodium on alumina (5 mol%) in 2 mL of methanol was stirred for two days in a sealed flask equipped with a balloon filled with hydrogen gas. The reaction mixture was filtered over a pad of celite, rinsed with 5 mL of methanol and the solvent was removed *in vacuo*. The crude product was purified by preparative HPLC (water/acetonitrile (97:3)) to obtain compound **325** as two separate diastereomers (13 mg DIA1, 13 mg DIA2, combined Y = 42%).

325a **325b**

Spectral data for DIA1 (**325a** or **325b**):

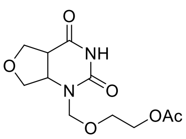
^{1}H NMR (400 MHz, DMSO-d$_6$): δ = 3.13-3.21 (m, 1H, CHC(O)), 3.32-3.4 (t, *J* = 8.4 Hz, 1H, OCH$_a$H$_b$CHN), 3.43-3.55 (m, 2H, CH$_2$OH), 3.68-3.74 (m, 1H, 4’-H), 3.75-3.83 (m, 2H, 2’-H, OCH$_a$H$_b$CHC(O)), 3.88 (dd, *J* = 2.3 Hz, *J* = 5.2 Hz, 1H, 3’-H), 3.97 (t, *J* = 8.4 Hz, 1H, OCH$_a$H$_b$CHN), 4.22 (dd, *J* = 0.8 Hz, *J* = 8.6 Hz, 1H, OCH$_a$H$_b$CHC(O)), 4.44 (q, *J* = 8.4 Hz, 1H, CHN), 4.70-5.39 (m, 3H, OH), 5.73 (d, *J* = 7.4 Hz, 1H, 1’-H), 10.6 (br. s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d$_6$): δ = 42.1 (CHC(O)), 50.0 (CHN), 61.7 (CH$_2$OH), 68.8 (OCH$_2$CHC(O)), 70.4 (3’-C), 71.0 (OCH$_2$CHN), 71.3 (2’-C), 83.9 (4’-C), 86.2 (1’-C), 152.0 (NC(O)NH), 170.5 (CHC(O)). IR: 1676, 1462, 1252, 1217, 1049, 1012, 405 cm^{-1}. MS (ESI, 70 eV): m/z (%) = 286.9 (100, [M – H]$^-$). HRMS: m/z [M – H]$^-$ calcd for $C_{11}H_{15}N_2O_7$: 287.0885; found: 287.0896. Colourless oil.

Spectral data for DIA2 (**325a** or **325b**):

^{1}H NMR (400 MHz, DMSO-d$_6$): δ = 3.15-3.25 (m, 2H, CHC(O), OCH$_a$H$_b$CHN), 3.41-3.53 (m, 2H, CH$_2$OH), 3.67 (q, *J* = 3.8 Hz, 1H, 4’-H), 3.80-3.90 (m, 2H, 3’-H, OCH$_a$H$_b$CHC(O)), 3.98-4.08 (m, 2H, 2’-H, OCH$_a$H$_b$CHN), 4.13 (dd, *J* = 1.4 Hz, *J* = 8.6 Hz, 1H, OCH$_a$H$_b$CHC(O)), 4.21 (q, *J* = 8.1 Hz, 1H, CHN), 4.60-5.50 (m, 3H, OH), 5.74 (d, *J* = 6.4 Hz, 1H, 1’-H), 10.6 (br. s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d$_6$): δ = 42.4 (CHC(O)), 51.0 (CHN), 61.5 (CH$_2$OH), 68.9 (OCH$_2$CHC(O)), 70.3 (2’-C, 3’-C), 71.7 (OCH$_2$CHN), 83.7 (4’-C), 87.4 (1’-C), 152.0 (NC(O)NH), 170.8 (CHC(O)). IR: 1674, 1244, 1223, 1051, 1013, 403 cm^{-1}. MS (ESI, 70 eV): m/z (%) = 287.0 (100, [M – H]$^-$). HRMS: m/z [M – H]$^-$ calcd for $C_{11}H_{15}N_2O_7$: 287.0885; found: 287.0898. Colourless oil.

1-((2-Acetoxyethoxy)methyl)tetrahydrofuro[3,4-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (326)

A suspension of 100 mg of **315** (0.37 mmol; 1 eq.) and 38 mg of 5 wt% rhodium on alumina (5 mol%) in 3 mL of dry methanol was stirred for one day in a sealed flask equipped with a balloon filled with hydrogen gas. The reaction mixture was filtered over a pad of celite, rinsed with 5 mL of methanol and the solvent was removed *in vacuo*. The residue was purified by column chromatography over silica (dichloromethane/methanol, gradient from 0% methanol to 10% methanol over 20 column volumes) to obtain compound **326** (mixture of cis-isomers) as a colourless oil (90 mg, Y = 89%).

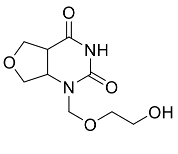


326

^{1}H NMR (400 MHz, MeOH-d$_4$): δ = 2.05 (s, 3H), 3.32-3.40 (m, 1H), 3.64 (dd, *J* = 7.4 Hz, *J* = 9.0 Hz, 1H), 3.67-3.78 (m, 2H), 4.06 (dd, *J* = 7.1 Hz, *J* = 8.9 Hz, 1H), 4.11 (dd, *J* = 7.4 Hz, *J* = 9.0 Hz, 1H), 4.14-4.34 (m, 4H), 4.78 (d, *J* = 10.6 Hz, 1H), 5.19

(d, J = 10.6 Hz, 1H). ^{13}C NMR (100 MHz, MeOH-d$_4$): δ = 20.8, 43.8, 57.6, 64.5, 67.8, 70.9, 73.1, 77.9, 153.6, 172.3, 172.7. IR: 1694, 1472, 1246, 1094, 1043 cm^{-1}. MS (ESI, 70 eV): m/z (%) = 271.0 (100, [M – H]$^-$).

1-((2-Hydroxyethoxy)methyl)tetrahydrofuro[3,4-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (327)



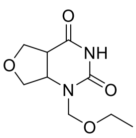
327

A solution of 90 mg of **326** (0.33 mmol; 1 eq.) in 10 mL of 7 M ammonia in methanol was stirred for 12 hours at room temperature. The solvent was evaporated, and the residue was purified by column chromatography over silica (dichloromethane/methanol, gradient from 0% to 10% methanol over 20 column volumes). The obtained product still contained acetamide, and was recrystallized from chloroform-diethyl ether to afford compound **327** (mixture of cis-isomers) as white crystals (25 mg, 33%).

^{1}H NMR (400 MHz, DMSO-d$_6$): δ = 3.30-3.34 (m, 1H, $\mathrm{C\underline{H}C(O)}$), 3.40-3.54 (m, 5H, $\mathrm{C\underline{H}_2C\underline{H}_2}$, $\mathrm{OC\underline{H}_aH_bCHN}$), 3.90-3.97 (m, 1H, $\mathrm{OC\underline{H}_aH_bCHC(O)}$), 3.97-4.04 (m, 1H, $\mathrm{OCH_a\underline{H}_bCHN}$), 4.05-4.11 (m, 1H, $\mathrm{OCH_a\underline{H}_bCHC(O)}$), 4.17-4.26 (m, 1H, $\mathrm{C\underline{H}N}$), 4.58-4.66 (m, 1H, $\mathrm{O\underline{H}}$), 4.75 (d, J = 10.4 Hz, 1H, $\mathrm{NC\underline{H}_aH_bO}$), 4.99 (d, J = 10.4 Hz, 1H, $\mathrm{NCH_a\underline{H}_bO}$), 10.60 (br. s, 1H, $\mathrm{N\underline{H}}$). ^{13}C NMR (100 MHz, DMSO-d$_6$): δ = 43.8 ($\mathrm{\underline{C}HC(O)}$), 57.3 ($\mathrm{\underline{C}HN}$), 62.1 ($\mathrm{\underline{C}H_2OH}$), 70.9 ($\mathrm{O\underline{C}H_2CHC(O)}$), 71.3 ($\mathrm{\underline{C}H_2CH_2OH}$), 73.0 ($\mathrm{O\underline{C}H_2CHN}$), 77.8 ($\mathrm{N\underline{C}H_2O}$), 153.6 ($\mathrm{N\underline{C}(O)NH}$), 172.3 ($\mathrm{CH\underline{C}(O)}$). IR: 3526, 3429, 3073, 2959, 2868, 1697, 1043, 411 cm^{-1}. MS (ESI, 70 eV): m/z (%) = 229.0 (100, [M – H]$^-$). Mp 100-101 °C.

1-(Ethoxymethyl)tetrahydrofuro[3,4-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (328)

To a solution of 100 mg of **323** (0.64 mmol; 1 eq.) in 2 mL of dry DMF was added 29 mg of sodium hydride (60% dispersion in mineral oil, 1.1 eq.) and the reaction mixture was stirred for fifteen minutes at room temperature. Then, 67 mg of chloromethyl ethyl ether was added (1.1 eq.) and the reaction mixture was stirred for one day at room temperature. The solvent was removed *in vacuo*, the residue was taken up in 20 mL of sat. aqueous ammonium chloride solution and extracted with dichloromethane (2 x 20 mL) and ethyl acetate (2 x 20 mL). The combined organic layers were dried over magnesium sulfate and the solvent was evaporated *in vacuo*. The crude product was purified by column chromatography over silica (dichloromethane/methanol, gradient from 0% methanol to 10% methanol over 15 column volumes) and preparative HPLC (water/acetonitrile (89:11)) to obtain compound **328** (mixture of cis-isomers) as a white solid (15 mg, Y = 11%).



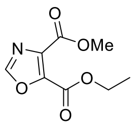
328

^{1}H NMR (400 MHz, DMSO-d$_6$): δ = 1.01-1.11 (m, 3H), 3.28-3.40 (m, 1H), 3.41-3.53 (m, 2H), 3.64-3.72 (m, 1H), 3.72-3.81 (m, 1H), 3.84-3.95 (m, 1H), 4.00-4.12 (m, 2H), 5.00-5.12 (m, 2H), 7.97 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d$_6$): δ = 15.1, 42.8, 49.8, 63.9, 68.5, 69.8, 73.9, 151.7, 169.7. IR: 3200, 3092, 2976, 2938, 2872, 1674, 1452, 1254, 1063, 561 cm^{-1}. MS (ESI, 70 eV): m/z (%) = 213.2 (100, [M – H]$^-$). Mp 99-100 °C.

2.2 Oxazolo[5,4-*d*]pyrimidine and oxazolo[4,5-*d*]pyrimidine derivatives

5-Ethyl 4-methyl oxazole-4,5-dicarboxylate (340a)

To a solution of 1.36 g of imidazole (20 mmol, 1 eq.) in 30 mL of dry tetrahydrofuran was added 8 mL of dry triethylamine (55 mmol, 2.75 eq.). The mixture was cooled down to 0 °C and a solution of 2.24 mL of ethyl 2-chloro-2-oxoacetate (20 mmol, 1 eq.) in 20 mL of dry tetrahydrofuran was added dropwise. After an additional 30 minutes of stirring at 0 °C, 1.82 mL of methyl 2-isocyanoacetate (20 mmol, 1 eq.) was added in one portion. The reaction mixture was stirred at 0 °C for one hour and then at reflux temperature for twelve hours. The solvent was removed *in vacuo*, and the crude product was purified by column chromatography over silica (ethyl acetate/hexane (1:4)) to obtain compound **340a** as a pale yellow oil (3.143 g, Y = 79%).



340a

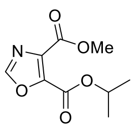
^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.42 (t, *J* = 7.1 Hz, 3H, $CH_2C\underline{H}_3$), 3.98 (s, 3H, $OC\underline{H}_3$), 4.45 (q, *J* = 7.1 Hz, 2H, $C\underline{H}_2$), 7.98 (s, 1H, $C\underline{H}$). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 14.0 ($CH_2\underline{C}H_3$), 52.9 ($O\underline{C}H_3$), 62.5 ($\underline{C}H_2CH_3$), 135.2 ($N\underline{C}_q$), 143.2 ($O\underline{C}_q$), 151.3 ($\underline{C}H$), 156.6 ($\underline{C}$(O)OEt), 160.6 ($\underline{C}$(O)OMe). IR: 1728, 1499, 1338, 1303, 1282, 1148, 1052, 762, 626 cm^{-1}. MS (ESI, 70 eV): m/z (%) = 200.0 (100, $[M + H]^+$). HRMS: m/z $[M + H]^+$ calcd for $C_8H_{10}NO_5$: 200.0553; found: 200.0558.

Isopropyl 2-chloro-2-oxoacetate (339b)

Isopropanol (3.83 mL, 0.05 mol, 1 eq.) was added dropwise to oxalyl chloride (9.5 mL, 0.1 mol, 2 eq.) at 0 °C. After addition, the mixture was allowed to warm up to room temperature, and stirring was continued for two hours. Excess oxalyl chloride was removed by distillation (1 atm, fraction with boiling point 60-80 °C). Further vacuum distillation (58 mbar, fraction with boiling point 64-66 °C) afforded compound **339b** as a colourless liquid (5.27 g, Y = 70%). All spectra were in accordance with reported spectral data.[210]

5-Isopropyl 4-methyl oxazole-4,5-dicarboxylate (340b)

To a solution of 1 g of imidazole (14.69 mmol, 1 eq.) in 20 mL of dry tetrahydrofuran was added 5.7 mL of dry triethylamine (2.75 eq.). The mixture was cooled down to 0 °C and a solution of 2.212 g of **339b** (1 eq.) in 15 mL of dry tetrahydrofuran was added dropwise. After an additional 30 minutes of stirring at 0 °C, 1.34 mL of methyl 2-isocyanoacetate (1 eq.) was added in one portion. The reaction mixture was stirred at 0 °C for one hour and then at reflux temperature for twelve hours. The solvent was removed *in vacuo* and the residue was dissolved in 25 mL of ethyl acetate and washed with 25 mL of water and 25 mL of brine. The organic layer was dried over magnesium sulfate and the solvent was removed *in vacuo*. The crude product was purified by column chromatography over silica (ethyl acetate/hexane (1:4), R_f = 0.275) to obtain compound **340b** as a pale yellow oil (2.889 g, Y = 92%).



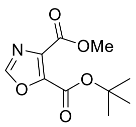
340b

^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.40 (d, *J* = 6.3 Hz, 6H), 3.97 (s, 3H), 5.30 (septet, *J* = 6.3 Hz, 1H), 7.98 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 21.7, 52.8, 70.7, 135.0, 143.4, 151.2, 156.2, 160.6. IR: 2985, 1725, 1589, 1370, 1307, 1283, 1158, 1101, 1050, 935, 844, 797, 763 cm^{-1}. MS (ESI, 70 eV): m/z (%) = 214.0 (23, $[M + H]^+$). HRMS: m/z $[M - H]^-$ calcd for $C_9H_{10}NO_5$: 212.0564; found: 212.0573.

5-(*tert*-Butyl) 4-methyl oxazole-4,5-dicarboxylate (340c)

tert-Butyl 2-chloro-2-oxoacetate (**339c**) was synthesized following a literature procedure, and was obtained as a colourless liquid in 50% yield.[147]

To a solution of 6.205 g of imidazole (0.09 mol, 1 eq.) in 130 mL of dry tetrahydrofuran was added 35 mL of dry triethylamine (2.75 eq.). The mixture was cooled down to 0 °C and a solution of 15 g of **339c** (1 eq.) in 90 mL of dry tetrahydrofuran was added dropwise. After an additional 30 minutes of stirring at 0 °C, 8.29 mL of methyl 2-isocyanoacetate (1 eq.) was added dropwise. The reaction mixture was stirred at 0 °C for one hour and then at reflux temperature for twelve hours. The solvent was removed *in vacuo* and the residue was dissolved in 100 mL of ethyl acetate and washed with 100 mL of water and 100 mL of brine. The organic layer was dried over magnesium sulfate and the solvent was removed *in vacuo*. The crude product was purified by column chromatography over silica (ethyl acetate/hexane (1:5), R_f = 0.3) to obtain compound **340c** as a pale yellow oil (19.68 g, Y = 95%).

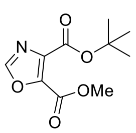


340c

^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.60 (s, 9H), 3.96 (s, 3H), 7.96 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 28.0, 52.8, 84.5, 134.4, 144.0, 150.9, 155.7, 160.8. IR: 2982, 1725, 1590, 1356, 1307, 1142, 1053, 765 cm^{-1}. MS (ESI, 70 eV): m/z (%) = 250 (7, $[M + Na]^+$). HRMS: m/z $[M – H]^-$ calcd for $C_{10}H_{12}NO_5$: 226.0721; found: 226.0720.

4-(*tert*-Butyl) 5-methyl oxazole-4,5-dicarboxylate (340d)

To a solution of 468 mg of imidazole (6.9 mmol, 1 eq.) in 15 mL of dry tetrahydrofuran was added 2.64 mL of dry triethylamine (2.75 eq.). The mixture was cooled down to 0 °C and a solution of 842 mg of methyl 2-chloro-2-oxoacetate (1 eq.) in 10 mL of dry tetrahydrofuran was added dropwise. After an additional 30 minutes of stirring at 0 °C, 1 mL of *tert*-butyl 2-isocyanoacetate (1 eq.) was added dropwise. The reaction mixture was stirred at 0 °C for one hour and then at reflux temperature for twelve hours. The solvent was removed *in vacuo* and the residue was dissolved in 100 mL of ethyl acetate and washed with 100 mL of water and 100 mL of brine. The organic layer was dried over magnesium sulfate and the solvent was removed *in vacuo*. The crude product was purified by column chromatography over silica (ethyl acetate/hexane (1:5), R_f = 0.32) to obtain compound **340d** as a pale yellow oil (1.354 g, Y = 87%).

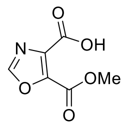


340d

^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.50-1.58 (m, 9H, $C(C\underline{H}_3)_3$), 3.87-3.93 (m, 3H, $OC\underline{H}_3$), 7.93 (s, 1H, $C\underline{H}$). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 27.9 ($C(\underline{C}H_3)_3$), 52.8 ($O\underline{C}H_3$), 83.5 ($\underline{C}(CH_3)_3$), 137.0 ($N\underline{C}_q$), 141.7 ($O\underline{C}_q$), 151.4 ($\underline{C}H$), 157.1 ($\underline{C}$(O)OMe), 159.4 ($\underline{C}$(O)O*t*-Bu). IR: 2530, 1714, 1580, 1429, 1306, 1159, 746 cm^{-1}. MS (ESI, 70 eV): m/z (%) = 250 (9, $[M + Na]^+$). HRMS: m/z $[M – H]^-$ calcd for $C_{10}H_{12}NO_5$: 226.0721; found: 226.0730.

5-(Methoxycarbonyl)oxazole-4-carboxylic acid (341)

To a solution of **340d** (2.525 g, 11.1 mmol, 1 eq.) in 23 mL of dry dichloromethane were added 4.44 mL of triethylsilane (2.5 eq.) and 11 mL of trifluoroacetic acid (13 eq.). After one hour of stirring at room temperature, the solvent was removed *in vacuo*, the residue was triturated in 6.5 mL of dry diethyl ether and filtered over a glass sintered filter. The filter cake was washed with 6.5 mL of dry diethyl ether and dried to obtain compound **341** as a white solid (1.787 g, Y = 94%).

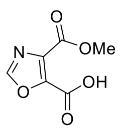


341

^{1}H NMR (400 MHz, DMSO-d_6): δ = 3.88 (s, 3H, OCH_3), 8.69 (s, 1H, CH), 13.79 (br. s, 1H, OH). ^{13}C NMR (100 MHz, DMSO-d_6): δ = 53.3 (OCH_3), 137.0 (NC_q), 141.0 (OC_q), 154.1 (CH), 157.4 (C(O)OMe), 162.2 (C(O)OH). IR: 3118, 2971, 1731, 1607, 1281, 1129, 1051, 732, 716 cm^{-1}. MS (ESI, 70 eV): m/z (%) = 172.0 (100, [M + H]$^+$). HRMS: m/z [M + H]$^+$ calcd for $C_6H_6NO_5$: 172.0240; found: 172.0238. Mp 136-137 °C.

4-(Methoxycarbonyl)oxazole-5-carboxylic acid (342)

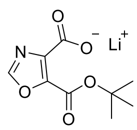
To a solution of **340c** (19.68 g, 86.6 mmol, 1 eq.) in 178 mL of dry dichloromethane were added 34.6 mL of triethylsilane (2.5 eq.) and 86.2 mL of trifluoroacetic acid (13 eq.). After one hour of stirring at room temperature, the solvent was removed *in vacuo*, the residue was triturated in 50 mL of dry diethyl ether and filtered over a glass sintered filter. The filter cake was washed with 50 mL of dry diethyl ether and dried to obtain compound **342** as a white solid (14.52 g, Y = 98%).



342

^{1}H NMR (400 MHz, DMSO-d_6): δ = 3.86 (s, 3H, OCH_3), 8.69 (d, J = 1.5 Hz, 1H, CH). ^{13}C NMR (100 MHz, DMSO-d_6): δ = 53.2 (OCH_3), 134.8 (NC_q), 143.2 (OC_q), 153.9 (CH), 158.0 (C(O)OH), 161.7 (C(O)OMe). IR: 2530, 1714, 1580, 1429, 1306, 1159, 746 cm^{-1}. MS (ESI, 70 eV): m/z (%) = 172.0 (87, [M + H]$^+$). HRMS: m/z [M + H]$^+$ calcd for $C_6H_6NO_5$: 172.0240; found: 172.0242. Mp 150-151 °C.

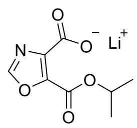
Lithium 5-(*tert*-butoxycarbonyl)oxazole-4-carboxylate (343)



343

To a stirred solution of 400 mg of **340c** (1.76 mmol, 1 eq.) in 25 mL of THF, a solution of 74 mg of LiOH monohydrate (1.76 mmol, 1 eq.) in 13 mL of water was added dropwise. The mixture was stirred at room temperature for one hour. THF was removed by rotary evaporation, and the remaining aqueous solution was washed two times with 10 mL of diethyl ether. After evaporation of the water phase and drying under high vacuum, compound 343 was obtained as a white, hygroscopic solid (333 mg, 86%). The purity of the compound was assessed by LCMS and NMR, but characterization of the compound was not performed.

Lithium 5-(isopropoxycarbonyl)oxazole-4-carboxylate (345)

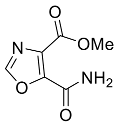


345

To a stirred solution of 506 mg of 340b (2.37 mmol, 1 eq.) in 25 mL of THF, a solution of 99.6 mg of LiOH monohydrate in 13 mL of water was added dropwise at 0 °C. The mixture was stirred at 0 °C for one hour and at room temperature for one more hour. The reaction was worked up according to the procedure for compound **343**, and resulted in a pale yellow oil (440 mg, 90%). Characterization of the compound was not performed.

Methyl 5-carbamoyloxazole-4-carboxylate (351)

To a suspension of 2.054 g of **342** (12 mmol, 1 eq.) in 80 mL of dry dichloromethane were added 1.22 mL of oxalyl chloride (1.2 eq.) and five drops of DMF. The reaction was stirred at room temperature for one hour and the solvent was removed *in vacuo*. The crude acyl chloride was taken up in 30 mL of dry dichloromethane and was added to a solution of 7.65 mL of hexamethyldisilazane (3 eq.) in 90 mL of dichloromethane at 0 °C. The reaction mixture was stirred at room temperature for twelve hours and the solvent was removed *in vacuo*. The crude product was dissolved in 30 mL of dry dichloromethane and 20 mL of trifluoroacetic acid (22 eq.) was added. After stirring at room temperature for one hour, the solvent was removed *in vacuo* and the crude product was suspended in 200 mL of sat. aqueous sodium bicarbonate solution. The suspension was extracted with ethyl acetate (3 x 200 mL). The combined organic layers were dried over magnesium sulfate and evaporated *in vacuo*. The crude product was triturated in 10 mL of acetone, triturated in 10 mL of hexane and dried to obtain product **351** as a white solid (1.79 g, Y = 88%).

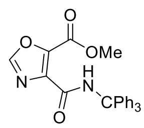


351

^{1}H NMR (400 MHz, DMSO-d$_6$): δ = 3.88 (s, 3H, OC$\underline{H}_3$), 8.21 (br. s, 1H, N$\underline{H}_a$H$_b$), 8.59 (br. s, 1H, NH$_a\underline{H}_b$), 8.65 (s, 1H, C$\underline{H}$). ^{13}C NMR (100 MHz, DMSO-d$_6$): δ = 53.3 (O$\underline{C}$H$_3$), 130.4 (N$\underline{C}_q$), 148.3 (O$\underline{C}_q$), 152.6 ($\underline{C}$H), 157.3 ($\underline{C}$(O)NH$_2$), 162.7 ($\underline{C}$(O)OMe). IR: 3333, 3183, 1708, 1682, 1318, 1300, 1170, 1055, 648 cm^{-1}. MS (ESI, 70 eV): m/z (%) = 171.0 (100, [M + H]$^+$). HRMS: m/z [M + H]$^+$ calcd for $C_6H_7N_2O_4$: 171.0400; found: 171.0400. Mp 184-185 °C (degrad.).

Methyl 4-(tritylcarbamoyl)oxazole-5-carboxylate (356)

To a suspension of **341** (100 mg, 0.58 mmol, 1 eq.) in 2 mL of dry dichloromethane was added 0.12 mL of oxalyl chloride (2.5 eq.) and one drop of DMF at 0 °C. After stirring for 15 minutes at 0 °C and two hours at room temperature, the solvent was removed *in vacuo* and the crude acyl chloride was taken up in 5 mL of dry dichloromethane and cooled down to 0 °C. Then, 0.41 mL of dry triethylamine (5 eq.) was added, followed by 152 mg of tritylamine (1 eq.). After stirring at room temperature for twelve hours, the solvent was removed in vacuo and the crude product was purified by column chromatography over silica (ethyl acetate/hexane (1:1), R_f = 0.4) to obtain compound **356** as a white solid (190 mg, Y = 79%).

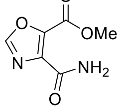


356

^{1}H NMR (400 MHz, CDCl$_3$): δ = 3.93 (s, 3H), 7.20-7.35 (m, 15H), 7.94 (s, 1H), 9.47 (br. s, 1H). ^{13}C NMR (100 MHz, CDCl$_3$): δ = 53.4, 71.2, 127.0, 127.9, 128.8, 139.9, 140.1, 144.3, 151.1, 157.3, 158.9. MS (ESI, 70 eV): m/z (%) = 411.1 (100, [M – H]$^-$). HRMS: m/z [M – H]$^-$ calcd for $C_{25}H_{19}N_2O_4$: 411.1350; found: 411.1361. mp 81-82 °C.

Methyl 4-carbamoyloxazole-5-carboxylate (357)

To a solution of 27 mg of **356** (0.065 mmol, 1 eq.) in 2 mL of dry dichloromethane was added 19 mg of triethylsilane (2.5 eq.) and 2 mL of trifluoroacetic acid. After stirring at room temperature for one hour, the solvent was removed *in vacuo* and the crude product was recrystallized from dichloromethane/hexane to obtain compound **357** as off-white crystals (9 mg, Y = 81%).



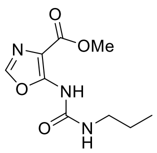
357

^{1}H NMR (400 MHz, DMSO-d$_6$): δ = 3.87 (s, 3H, OC$\underline{H}_3$), 7.83 (br. s, 1H, N$\underline{H}_a$H$_b$), 8.08 (br. s, 1H, NH$_a\underline{H}_b$), 8.67 (s, 1H, C$\underline{H}$). ^{13}C NMR (100 MHz, DMSO-d$_6$): δ = 53.2 (O$\underline{C}$H$_3$), 139.1 (O$\underline{C}_q$), 140.1 (N$\underline{C}_q$), 153.9 ($\underline{C}$H), 158.2 ($\underline{C}$(O)OMe), 161.6 ($\underline{C}$(O)NH$_2$). IR: 3325, 1674, 1632, 1531, 1493, 1449, 1306, 1202, 1051, 698, 910 cm^{-1}. MS (ESI, 70 eV): m/z (%) = 171.0 (100, [M + H]$^+$). HRMS: m/z [M + H]$^+$ calcd for $C_6H_7N_2O_4$: 171.0400; found: 171.0402. Mp 168-169 °C.

Representative procedure for compounds **350a-c,e-g**:

Methyl 5-(3-propylureido)oxazole-4-carboxylate (350a)

To a suspension of 200 mg of **351** (1.2 mmol, 1 eq.) in 10 mL of dry dichloromethane was added 1.136 g of PhI(OAc)$_2$ (3 eq.) and the reaction mixture was stirred at room temperature for two hours. Then, 417 mg of *n*-propylamine (6 eq.) was added, and the reaction mixture was stirred at room temperature for twelve hours. The reaction mixture was poured into a separation funnel, 10 mL of dichloromethane was added and the organic layer was washed with 15 mL of sat. aqueous ammonium chloride solution. The aqueous layer was extracted with dichloromethane (2 x 20 mL), the combined organic layers were dried over magnesium sulfate and the solvent was removed *in vacuo*. The crude product was purified by column chromatography over silica (dichloromethane/methanol, gradient from 0% methanol to 5% methanol over 10 column volumes, R_f = 0.43 (5% methanol in dichloromethane)) to afford a pale yellow product. After trituration in 1 mL of diethyl ether, compound **350a** was obtained as a white solid (156 mg, Y = 58%).

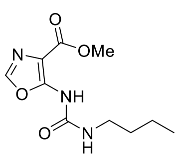


350a

^{1}H NMR (400 MHz, CDCl$_3$): δ = 0.98 (t, *J* = 7.2 Hz, 3H, CH$_2$C$\underline{H}_3$), 1.62 (sextet, *J* = 7.2 Hz, 2H, C$\underline{H}_2$CH$_3$), 3.31 (dt, *J* = 5.9 Hz, *J* = 7.2 Hz, 2H, NC$\underline{H}_2$), 3.91 (s, 3H, OC$\underline{H}_3$), 5.87 (br. s, 1H, N$\underline{H}$CH$_2$), 7.51 (s, 1H, C$\underline{H}$), 8.47 (br. s, 1H, C$_q$N$\underline{H}$C(O)). ^{13}C NMR (100 MHz, CDCl$_3$): δ = 11.3 (CH$_2\underline{C}$H$_3$), 23.0 ($\underline{C}$H$_2$CH$_3$), 42.5 (N$\underline{C}$H$_2$), 52.1 (O$\underline{C}$H$_3$), 108.5 (N$\underline{C}_q$), 142.1 ($\underline{C}$H), 151.1 (NH$\underline{C}$(O)NH), 152.2 (O$\underline{C}_q$), 163.3 ($\underline{C}$(O)OMe). IR: 3333, 2972, 1697, 1638, 1450, 1310, 1061, 785, 631 cm^{-1}. MS (ESI, 70 eV): m/z (%) = 225.9 (100, [M – H]$^-$). HRMS: m/z [M - H]$^-$ calcd for $C_9H_{12}N_3O_4$: 226.0833; found: 226.0838. Mp 167-168 °C.

Methyl 5-(3-butylureido)oxazole-4-carboxylate (350b)

Compound **350b** was synthesized and purified following the representative procedure, using 200 mg of **351**, 1.136 g of PhI(OAc)$_2$ and 516 mg of *n*-butylamine. R_f = 0.41 (5% methanol in dichloromethane). Compound **350b** was obtained as a white solid (159 mg, Y = 56%).

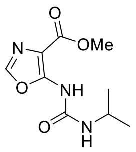


350b

^{1}H NMR (400 MHz, CDCl$_3$): δ = 0.95 (t, *J* = 7.4 Hz, 3H), 1.40 (sextet, *J* = 7.4 Hz, 2H), 1.58 (pentet, *J* = 7.4 Hz, 2H), 3.30-3.38 (m, 2H), 3.90 (s, 3H), 5.90 (br. s, 1H), 7.51 (s, 1H), 8.51 (br. s, 1H). ^{13}C NMR (100 MHz, CDCl$_3$): δ = 13.7, 20.0, 31.8, 40.5, 52.0, 108.5, 142.1, 151.2, 152.2, 163.3. IR: 3316, 1667, 1636, 1450, 1215, 1084, 1053, 642 cm^{-1}. MS (ESI, 70 eV): m/z (%) = 239.6 (100, [M – H]$^-$). HRMS: m/z [M + H]$^+$ calcd for $C_{10}H_{16}N_3O_4$: 242.1135; found: 242.1133. Mp 145-146 °C.

Methyl 5-(3-isopropylureido)oxazole-4-carboxylate (350c)

Compound **350c** was synthesized and purified following the representative procedure, using 200 mg of **351**, 1.136 g of $PhI(OAc)_2$ and 418 mg of isopropylamine. R_f = 0.39 (5% methanol in dichloromethane). Compound **350c** was obtained as a white solid (173 mg, Y = 65%).

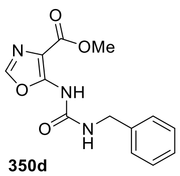


350c

^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.25 (d, *J* = 6.4 Hz, 6H), 3.90 (s, 3H), 4.04 (octet, *J* = 6.4 Hz, 1H), 5.69 (d, *J* = 6.4 Hz, 1H), 7.51 (s, 1H), 8.47 (br. s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 22.9, 43.0, 52.0, 108.6, 142.2, 150.3, 152.3, 163.3. IR: 3327, 1707, 1634, 1499, 1314, 1090, 629 cm^{-1}. MS (ESI, 70 eV): m/z (%) = 250.2 (73, $[M + Na]^+$). HRMS: m/z $[M + H]^+$ calcd for $C_9H_{14}N_3O_4$: 228.0979; found: 228.0973. Mp 177-178 °C.

Methyl 5-(3-benzylureido)oxazole-4-carboxylate (350d)

To a suspension of 200 mg of **351** (1.2 mmol, 1 eq.) in 30 mL of dry dichloromethane was added 2.272 g of $PhI(OAc)_2$ (6 eq.) and the reaction mixture was stirred at reflux temperature for two and a half hours. The reaction was cooled down to 0 °C, 0.771 mL of benzylamine (6 eq.) was added, the reaction mixture was allowed to warm up to room temperature and stirred for twelve more hours. The reaction mixture was poured into a separation funnel and the organic layer was washed with 20 mL of sat. aqueous ammonium chloride solution. The aqueous layer was extracted with dichloromethane (2 x 20 mL), the combined organic layers were dried over magnesium sulfate and the solvent was removed *in vacuo*. The crude product was purified by column chromatography over silica (dichloromethane/methanol, gradient from 0% methanol to 5% methanol over 10 column volumes) to afford a pale yellow product. After trituration in 1 mL of diethyl ether and subsequent recrystallization from methanol, compound **350d** was obtained as white crystals (49 mg, Y = 15%).

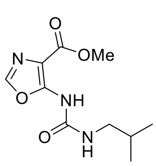


350d

^{1}H NMR (400 MHz, DMSO-d_6): δ = 3.78 (s, 3H), 4.31 (d, *J* = 5.4 Hz, 2H), 7.22-7.41 (m, 5H), 7.69-7.78 (m, 1H), 8.13 (s, 1H), 9.23 (br. s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ = 43.5, 51.8, 111.8, 127.5, 127.7, 128.9, 139.8, 145.8, 152.1, 152.5, 162.5. IR: 3262, 1626, 1537, 1206, 1036, 669 cm^{-1}. MS (ESI, 70 eV): m/z (%) = 274.0 (100, $[M – H]^-$). HRMS: m/z $[M + H]^+$ calcd for $C_{13}H_{14}N_3O_4$: 276.0979; found: 276.0979. Mp 176-177 °C.

Methyl 5-(3-isobutylureido)oxazole-4-carboxylate (350e)

Compound **350e** was synthesized and purified following the representative procedure, using 200 mg of **351**, 1.136 g of $PhI(OAc)_2$ and 516 mg of isobutylamine. R_f = 0.4 (5% methanol in dichloromethane). Compound **350e** was obtained as a white solid (144 mg, Y = 51%).

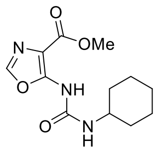


350e

^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.97 (d, *J* = 6.7 Hz, 6H), 1.86 (nonet, *J* = 6.7 Hz, 1H), 3.14-3.20 (m, 2H), 3.91 (s, 3H), 5.93 (br. s, 1H), 7.51 (s, 1H), 8.51 (br. s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 20.0, 28.6, 48.1, 52.0, 108.5, 142.1, 151.2, 152.2, 163.3. IR: 3331, 2965, 1697, 1636, 1568, 1206, 1081, 627 cm^{-1}. MS (ESI, 70 eV): m/z (%) = 240.3 (100, $[M – H]^-$). HRMS: m/z $[M + H]^+$ calcd for $C_{10}H_{16}N_3O_4$: 242.1135; found: 242.1137. Mp 151-152 °C.

Methyl 5-(3-cyclohexylureido)oxazole-4-carboxylate (350f)

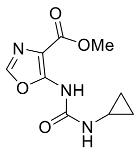
Compound **350f** was synthesized and purified following the representative procedure, using 200 mg of **351**, 1.136 g of PhI(OAc)$_2$ and 700 mg of cyclohexylamine. R_f = 0.55 (5% methanol in dichloromethane). Compound **350f** was obtained as a white solid (199 mg, Y = 63%).


350f

^{1}H NMR (400 MHz, CDCl$_3$): δ = 1.14-1.30 (m, 3H), 1.32-1.48 (m, 2H), 1.56-1.69 (m, 1H), 1.69-1.80 (m, 2H), 1.95-2.07 (m, 2H), 3.64-3.80 (m, 1H), 3.91 (s, 3H), 5.63 (br. s, 1H), 7.51 (s, 1H), 8.40 (br. s, 1H). ^{13}C NMR (100 MHz, CDCl$_3$): δ = 24.7, 25.4, 33.2, 49.7, 52.1, 108.3, 142.0, 150.1, 152.2, 163.3. IR: 3296, 1661, 1634, 1449, 1209, 1084, 1053, 637 cm^{-1}. MS (ESI, 70 eV): m/z (%) = 268.1 (100, [M + H]$^+$). HRMS: m/z [M + H]$^+$ calcd for $C_{12}H_{18}N_3O_4$: 268.1292; found: 268.1290. Mp 192-193 °C.

Methyl 5-(3-cyclopropylureido)oxazole-4-carboxylate (350g)

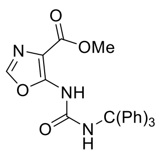
Compound **350g** was synthesized and purified following the representative procedure, using 200 mg of **351**, 1.136 g of PhI(OAc)$_2$ and 403 mg of cyclopropylamine. R_f = 0.4 (5% methanol in dichloromethane). After trituration in diethyl ether, the obtained product was purified by reversed phase column chromatography on C18 silica (water/acetonitrile, gradient from 0% acetonitrile to 100% acetonitrile over 20 column volumes) and subsequent recrystallization from acetonitrile to obtain compound **350g** as white crystals (50 mg, Y = 19%).


350g

^{1}H NMR (400 MHz, CDCl$_3$): δ = 0.60-0.83 (m, 2H), 0.84-1.08 (m, 2H), 2.65-2.76 (m, 1H), 3.93 (s, 3H), 5.68 (br. s, 1H), 7.55 (s, 1H), 9.37 (br. s, 1H). ^{13}C NMR (100 MHz, DMSO-d$_6$): δ = 6.2, 22.4, 51.4, 111.0, 145.2, 151.7, 152.6, 162.1. MS (ESI, 70 eV): m/z (%) = 226.1 (100, [M + H]$^+$). HRMS: m/z [M + H]$^+$ calcd for $C_9H_{12}N_3O_4$: 226.0822; found: 226.0822. Mp 171-172 °C.

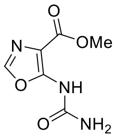
Methyl 5-(3-tritylureido)oxazole-4-carboxylate (350h)

To a suspension of 200 mg of **351** (1.2 mmol, 1 eq.) in 25 mL of dry dichloromethane was added 2.272 g of PhI(OAc)$_2$ (6 eq.) and the reaction mixture was stirred at reflux temperature for two and a half hours. The reaction was cooled down to 0 °C, 1.829 g of tritylamine (6 eq.) was added and the reaction mixture was stirred at 0 °C for 30 minutes. The reaction was quenched by addition of 20 mL of a sat. sodium thiosulfate solution and stirred at 0 °C for 30 minutes. The reaction mixture was poured into a separation funnel and the organic layer was separated. The aqueous layer was extracted with dichloromethane (2 x 20 mL), the combined organic layers were dried over magnesium sulfate and the solvent was removed *in vacuo*. The crude product was purified by column chromatography over silica (dichloromethane/methanol, gradient from 0% methanol to 5% methanol over 10 column volumes, R_f = 0.5 (5% methanol in dichloromethane)) to afford a white solid. After trituration in 1 mL of diethyl ether, compound **350h** was obtained as a white solid (155 mg, Y = 31%).


350h

^{1}H NMR (400 MHz, DMSO-d$_6$): δ = 3.78 (s, 3H), 7.05-7.45 (m, 15H), 8.01 (s, 1H), 8.47 (s, 1H), 9.70 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d$_6$): δ = 51.8, 69.7, 111.2, 127.1, 128.2, 128.9, 145.2, 145.4, 151.1, 152.1, 162.6. IR: 1632, 1533, 1306, 1202, 1051, 698 cm^{-1}. MS (ESI, 70 eV): m/z (%) = 426.0 (100, [M – H]$^-$). HRMS: m/z [M + H]$^+$ calcd for $C_{25}H_{22}N_3O_4$: 428.1605; found: 428.1585. Mp 238-239 °C.

Methyl 5-ureidooxazole-4-carboxylate (350i)



350i

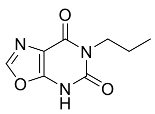
To a solution of 543 mg of **350h** (1.27 mmol, 1 eq.) in 10 mL of dry dichloromethane were added 369 mg of triethylsilane (2.5 eq.) and 1.26 mL of trifluoroacetic acid (13 eq.). After stirring at room temperature for one hour, the solvent was evaporated *in vacuo* and an off-white solid was obtained. The crude material was suspended in 5 mL of dichloromethane and triturated. Then, 15 mL of hexane was added and the solvent was decanted. Next, the solids were suspended in 5 mL of acetone and triturated. Then, 15 mL of hexane was added and the solvent was decanted. After drying under high vacuum, compound **350i** was obtained as a white solid (193 mg, 82%).

^{1}H NMR (400 MHz, DMSO-d$_6$): δ = 3.78 (s, 3H, OC$\underline{H}_3$), 6.71 (s, 2H, N$\underline{H}_2$), 8.10 (s, 1H, C$\underline{H}$), 9.07 (br. s, 1H, N$\underline{H}$). ^{13}C NMR (100 MHz, DMSO-d$_6$): δ = 51.4 (O$\underline{C}$H$_3$), 111.3 ($\underline{C}_q$C(O)OMe), 145.3 ($\underline{C}$H), 151.8 ($\underline{C}_q$NHC(O)), 152.7 (NH$\underline{C}$(O)NH$_2$), 162.1 ($\underline{C}$(O)OMe). IR: 3345, 3184, 3142, 1730, 1672, 1611, 1327, 1063, 635, 610 cm^{-1}. MS (ESI, 70 eV): m/z (%) = 184.1 (87, [M – H]$^-$). Mp 213-214 °C (degrad.).

Representative procedure for the synthesis of compounds **337a-d**:

6-Propyloxazolo[5,4-*d*]pyrimidine-5,7(4*H*,6*H*)-dione (337a)

To a suspension of 50 mg of **350a** (0.22 mmol, 1 eq.) in 0.8 mL of dry methanol was added 35 mg of sodium methoxide (2.9 eq.). The reaction mixture was stirred at room temperature for 27 days. The solvent was removed *in vacuo*, and the residue was dissolved in 0.5 mL of ice cold water. The solution was acidified to pH = 5~6 by dropwise addition of 1N aqueous sulfuric acid solution at 0 °C. The precipitate was filtered off and washed with 1 mL of ice cold water and dried under high vacuum to obtain compound **337a** as an off-white solid (20 mg, Y = 46%).



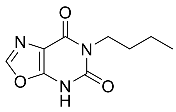
337a

^{1}H NMR (400 MHz, DMSO-d$_6$): δ = 0.86 (t, *J* = 7.5 Hz, 3H, C$\underline{H}_3$), 1.54 (pentet, *J* = 7.5 Hz, 2H, C$\underline{H}_2$CH$_3$), 3.74-3.81 (m, 2H, NC$\underline{H}_2$), 8.31 (s, 1H, C$\underline{H}$), 13.30 (br. s, 1H, N$\underline{H}$). ^{13}C NMR (100 MHz, DMSO-d$_6$): δ = 11.6 ($\underline{C}$H$_3$), 21.1 ($\underline{C}$H$_2$CH$_3$), 42.3 (N$\underline{C}$H$_2$), 110.3 (N$\underline{C}_q$), 147.1 ($\underline{C}$H), 150.3 (NH$\underline{C}$(O)N), 155.0 (O$\underline{C}_q$), 157.2 (C$_q\underline{C}$(O)). IR: 1722, 1643, 1555, 1055, 1011 cm^{-1}. MS (ESI, 70 eV): m/z (%) = 196.0 (100, [M + H]$^+$). HRMS: m/z [M – H]$^-$ calcd for $C_8H_8N_3O_3$: 194.0571; found: 194.0566. Mp 170-171 °C (degrad.).

6-Butyloxazolo[5,4-*d*]pyrimidine-5,7(4*H*,6*H*)-dione (337b)

Compound **337b** was synthesized following the representative procedure, using 70 mg of **350b** (0.29 mmol) and 46 mg of sodium methoxide, and was obtained as an off-white solid (31 mg, Y = 51%).

^{1}H NMR (400 MHz, DMSO-d$_6$): δ = 0.89 (t, *J* = 7.4 Hz, 3H), 1.28 (sextet, *J* = 7.4 Hz, 2H), 1.49 (pentet, *J* = 7.4 Hz, 2H), 3.79 (t, *J* = 7.4 Hz, 2H), 8.19 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d$_6$): δ = 13.7, 19.6,

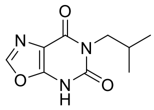


337b

29.6, 40.2, 109.2, 146.1, 151.1, 156.6, 157.1. IR: 3136, 3082, 2963, 2870, 1643, 1555, 1013, 752, 621, 525, 492 cm^{-1}. MS (ESI, 70 eV): m/z (%) = 210.1 (100, $[M + H]^+$). Mp 169-170 °C (degrad.).

6-Isobutyloxazolo[5,4-*d*]pyrimidine-5,7(4*H*,6*H*)-dione (337c)

Compound **337c** was synthesized following the representative procedure, using 28.5 mg of **350c** (0.12 mmol) and 18.5 mg of sodium methoxide, and was obtained as an off-white solid (13 mg, Y = 53%).

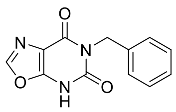


337c

^1H NMR (400 MHz, DMSO-d_6): δ = 0.78-0.92 (m, 6H), 1.93-2.10 (m, 1H), 3.60-3.72 (m, 2H), 8.31 (s, 1H), 13.29 (br. s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ = 19.9, 26.6, 47.0, 109.8, 146.6, 150.0, 154.4, 157.0. IR: 3134, 3084, 3053, 2963, 1726, 1653, 1555, 754, 623, 540 cm^{-1}. MS (ESI, 70 eV): m/z (%) = 210.1 (100, $[M + H]^+$). Mp 170-171 °C (degrad.).

6-Benzyloxazolo[5,4-*d*]pyrimidine-5,7(4*H*,6*H*)-dione (337d)

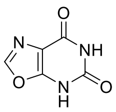
Compound **337d** was synthesized following the representative procedure, using 31 mg of **350d** (0.11 mmol) and 18 mg of sodium methoxide, and was obtained as an off-white solid (9 mg, Y = 33%).



337d

^1H NMR (400 MHz, DMSO-d_6): δ = 5.01 (s, 2H), 7.15-7.35 (m, 5H), 8.31 (s, 1H), 13.4 (br. s, 1H). MS (ESI, 70 eV): m/z (%) = 242.2 (100, $[M - H]^-$).

Oxazolo[5,4-*d*]pyrimidine-5,7(4*H*,6*H*)-dione (337e)



337e

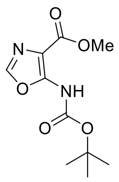
To a suspension of 50 mg of **350i** (0.27 mmol, 1 eq.) in 1.8 mL of dry methanol was added 42 mg of sodium methoxide (2.9 eq.). The reaction mixture was stirred at room temperature for 15 days. The solvent was removed *in vacuo*, and the residue was taken up in 2 mL of ice cold water. The suspension was acidified to pH = 5~6 by dropwise addition of 2N aqueous HCl solution at 0 °C. The precipitate was filtered off and washed with 1 mL of ice cold water and dried under high vacuum to obtain compound **337e** as an off-white solid (20 mg, Y = 48%).

^1H NMR (400 MHz, DMSO-d_6): δ = 8.07 (s, 1H, C$\underline{H}$), 10.49 (br. s, 1H, N$\underline{H}$). ^{13}C NMR (100 MHz, DMSO-d_6): δ = 109.2 (N$\underline{C}_q$C(O)), 145.6 ($\underline{C}$H), 153.2 (NH$\underline{C}$(O)NH), 158.0 (O$\underline{C}_q$NH), 160.9 (N$C_q\underline{C}$(O)). IR: 1682, 1630, 1609, 1281, 758, 625, 525, 482 cm^{-1}. MS (ESI, 70 eV): m/z (%) = 152.1 (100, $[M – H]^-$). Mp 249-250 °C (degrad.).

Methyl 5-((*tert*-butoxycarbonyl)amino)oxazole-4-carboxylate (369)

To a solution of 100 mg of **350a** (0.44 mmol, 1 eq.) in 5 mL of dry THF were added 0.08 mL of triethylamine (1.3 eq.) and 27 mg of 4-(dimethylamino)pyridine (0.5 eq.) under an inert atmosphere. The mixture was cooled down to 0 °C and a solution of 125 mg of di-*tert*-butyl dicarbonate (1.3 eq.) in

1 mL of dry THF was added dropwise to the reaction. The reaction mixture was warmed up to room temperature and stirred for two hours. Then, 10 mL of water was added and the mixture was extracted with ethyl acetate (3 x 20 mL). The combined organic layers were washed with brine (10 mL), dried over magnesium sulfate and evaporated *in vacuo*. The crude product was purified by column chromatography over silica (ethyl acetate/hexane (1:3), R_f = 0.31) to obtain compound **369** as white crystals (86 mg, Y = 81%).

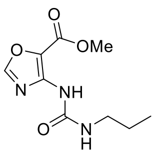


369

^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.54 (s, 9H), 3.93 (s, 3H), 7.54 (s, 1H), 8.44 (br. s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 28.0, 52.1, 83.3, 110.1, 143.4, 149.3, 151.9, 163.5. IR: 3121, 1705, 1626, 1312, 1148, 642 cm^{-1}. MS (ESI, 70 eV): m/z (%) = 240.9 (100, $[M – H]^-$). HRMS: m/z $[M – H]^-$ calcd for $C_{10}H_{13}N_2O_5$: 241.0830; found: 241.0830. White crystals, mp 81-82 °C.

Methyl 4-(3-propylureido)oxazole-5-carboxylate (370a)

Compound **370a** was synthesized and purified following the representative procedure for compounds **350a-c,e-g**, using 100 mg of **357**, 568 mg of $PhI(OAc)_2$ and 209 mg of *n*-propylamine. R_f = 0.43 (ethyl acetate/hexane (1:1)). Compound **370a** was obtained as a colourless oil (82 mg, Y = 61%).

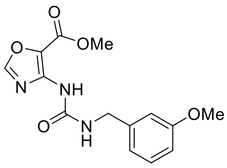


370a

^{1}H NMR (400 MHz, MeOH-d_4): δ = 0.90-1.02 (m, 3H, $CH_2C\underline{H}_3$), 1.53-1.66 (m, 2H, $C\underline{H}_2CH_3$), 3.21-3.30 (m, 2H, $NC\underline{H}_2$), 3.93 (s, 3H, $OC\underline{H}_3$), 8.30 (s, 1H, $C\underline{H}$). ^{13}C NMR (100 MHz, MeOH-d_4): δ = 11.7 ($CH_2\underline{C}H_3$), 24.0 ($\underline{C}H_2CH_3$), 42.9 ($NH\underline{C}H_2$), 52.5 ($O\underline{C}H_3$), 124.2 ($N\underline{C}_q$), 146.1 ($O\underline{C}q$), 154.0 ($\underline{C}H$), 155.5 ($NH\underline{C}(O)NH$), 160.2 ($\underline{C}(O)OMe$). IR: 3345, 3094, 2961, 2874, 1678, 1624, 1549, 1447, 1246, 1148 cm^{-1}. MS (ESI, 70 eV): m/z (%) = 226.2 (100, $[M – H]^-$).

Methyl 4-(3-(3-methoxybenzyl)ureido)oxazole-5-carboxylate (370c)

To a suspension of 300 mg of **341** (1.75 mmol, 1 eq.) in 5 mL of dry dichloromethane were added 0.18 mL of oxalyl chloride and one drop of DMF at room temperature. The reaction was stirred for one and a half hours at room temperature, and the solvent was removed *in vacuo*. The crude acyl chloride was dissolved in 5 mL of dry acetone and a solution of 171 mg of sodium azide (1.5 eq.) in 1 mL of water was added dropwise to the reaction mixture at 0 °C. After stirring for one hour at 0 °C, 10 mL of water was added and the mixture was extracted with ethyl acetate (2 x 10 mL). The combined organic layers were dried over magnesium sulfate and evaporated *in vacuo* at low temperature (20 to 25 °C) to afford the pure acyl azide **372** (316 mg, Y = 92%). A solution of 116 mg of **372** (0.51 mmol, 1 eq.) in 5 mL of toluene was heated at 70 °C for one hour and fifteen minutes, cooled down to room temperature and 163 mg of 3-methoxybenzylamine was added to the reaction mixture (2 eq.). After stirring for twelve hours at room temperature, the solvent was evaporated *in vacuo* and the crude product was purified by column chromatography over silica (ethyl acetate/hexane (1:1), R_f = 0.27) to obtain compound **370c** as a colourless oil (63 mg, Y = 35%).



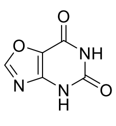
370c

^{1}H NMR (400 MHz, MeOH-d_4): δ = 3.76 (s, 3H), 3.90 (s, 3H), 4.44 (s, 2H), 6.76-6.83 (m, 1H), 6.87-6.97 (m, 2H), 7.18-7.25 (m, 1H), 8.22 (s, 1H). ^{13}C NMR (100 MHz, MeOH-d_4): δ = 44.8, 52.5, 55.6, 113.7, 114.0, 120.6, 124.5, 130.7, 141.6, 145.9, 154.0, 155.5, 160.2, 161.4. IR: 3329, 2955, 1678, 1624, 1445, 1240, 1144, 1045, 758, 610 cm^{-1}. MS (ESI, 70 eV): m/z (%) = 304.2 (100, [M – H]$^-$).

Oxazolo[4,5-*d*]pyrimidine-5,7(4*H*,6*H*)-dione (333a)

A suspension of 100 mg of **372** (0.51 mmol, 1 eq.) and 67 mg of 1*H*-benzotriazole (1.1 eq.) in 3 mL of dry toluene was heated at 70 °C for 1.5 hours. After a few minutes, the suspension turned into a clear solution, and after 1.5 hours, a white precipitate is observed in the reaction. The solvent was evaporated, and an off-white solid was obtained. The solid was taken up in 5 mL of a 7M solution of ammonia in methanol, and stirred at room temperature for fifteen minutes. The solvent was removed *in vacuo*, and the residue was triturated in 5 mL of diethyl ether and decanted. This washing process was repeated two more times, and the product was dried under high vacuum. 50 mg of an off-white powder was obtained. This product still contained residual 1*H*-benzotriazole and a small amount of side products. A small sample was recrystallized from absolute ethanol for characterization purposes (**370b**, *vide infra*).

43 mg of crude **370b** was suspended in 1.2 mL of absolute ethanol, and 0.23 mL of a 1M aq. NaOH solution was added (1 eq.). The reaction mixture was heated at 80 °C for two hours. After cooling down to room temperature, the solvent was removed *in vacuo*. The residue was taken up in 1 mL of ice cold water and acidified to pH 5~6 with 1M aqueous H_2SO_4. The water layer was extracted with ethyl acetate (3x 5mL). The organic layers were combined, washed with 5 mL of brine, dried over magnesium sulfate and evaporated *in vacuo*. The crude product was purified by preparative HPLC (isocratic eluent mixture of acetonitrile/water 3:97). Compound **333a** was obtained as a white powder (4 mg, 6% yield from **372** to **333a**).

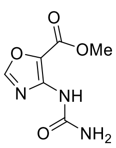


333a

^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.45 (s, 1H, CH), 10.17 (br. s, 1H, NH). IR: 1688, 1582, 1335, 1132, 775, 754, 598, 561, 528, 480 cm^{-1}. MS (ESI, 70 eV): m/z (%) = 152.1 (100, [M – H]$^-$).

Methyl 4-ureidooxazole-5-carboxylate (370b)

White crystals from absolute ethanol (see procedure for **333a**).

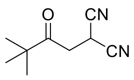


370b

^{1}H NMR (400 MHz, DMSO-d_6): δ = 3.83 (s, 3H, OCH_3), 6.92 (br. s, 2H, NH_2), 8.12 (br. s, 1H, NH), 8.56 (s, 1H, CH). ^{13}C NMR (100 MHz, DMSO-d_6): δ = 51.8 (OCH$_3$), 123.4 (NC_qNHC(O)), 144.4 (OC_qC(O)), 153.3 (CH), 153.3 (NHC(O)NH), 158.3 (OC$_q$$C$(O)). IR: 3404, 3377, 3217, 3115, 1719, 1688, 1632, 1609, 1315, 1288, 1142, 754, 610, 484, 463 cm^{-1}. MS (ESI, 70 eV): m/z (%) = 186.0 (100, [M + H]$^+$).

2-(3,3-Dimethyl-2-oxobutyl)malononitrile (382)

Chloropinacolone (5 g, 37 mmol, 1 eq.) was dissolved in 25 mL of methanol and a mixture of malononitrile (2.60 g, 1.06 eq.) and triethylamine (5.18 mL, 1 eq.) in 25 mL of methanol was added dropwise to the reaction mixture under an inert atmosphere at such a rate as to maintain the reaction temperature below 40 °C. The reaction mixture was stirred at room temperature for two hours and the solvent was evaporated *in vacuo*. The residue was taken up into 20 mL of dichloromethane, washed with 20 mL of brine, dried over magnesium sulfate, filtered over a plug of silica and evaporated to dryness to obtain compound **382** as pale brown crystals (5.223 g, Y = 86%).

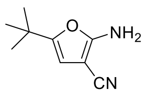


382

^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.22 (s, 9H, $C(C\underline{H}_3)_3$), 3.28 (d, *J* = 6.9 Hz, 2H, $C\underline{H}_2$), 4.22 (t, *J* = 6.9 Hz, 1H, $C\underline{H}$).

2-Amino-5-(*tert*-butyl)furan-3-carbonitrile (376)

A solution of 500 mg of **382** (3 mmol, 1 eq.) in 3 mL of dry tetrahydrofuran and 1 mL of triethylamine was heated at 90 °C for one hour under an inert atmosphere in a microwave reactor. The solvent was evaporated *in vacuo* and the residue was dissolved in 10 mL of dichloromethane and washed with 10 mL of brine. The aqueous layer was extracted again with 10 mL of dichloromethane. The combined organic layers were dried over magnesium sulfate, filtered over a plug of silica and evaporated to dryness. The crude product was recrystallized from hexane to obtain compound **376** as pale brown crystals (425 mg, Y = 85%).

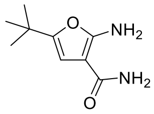


376

^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.20 (s, 9H, $C(C\underline{H}_3)_3$), 4.57 (br. s, 2H, $N\underline{H}_2$), 5.83 (s, 1H, $C\underline{H}$).

2-Amino-5-(*tert*-butyl)furan-3-carboxamide (375)

To 5.7 mL of concentrated sulfuric acid was added 1.49 g of **376** (9 mmol, 1 eq.) and the mixture was heated at 60 °C for 30 minutes. The reaction mixture was cooled down to 0 °C and 20 g of crushed ice was added to the reaction. The pH of the mixture was adjusted to pH = 9 by dropwise addition of a 28% ammonia in water solution. The formed precipitate was filtered off, washed with 10 mL of water and dried under high vacuum to obtain compound **375** as a dark purple powder (1.276 g, Y = 77%).



375

^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.22 (s, 9H, $C(C\underline{H}_3)_3$), 5.20 (br. s, 2H, $N\underline{H}_2$), 5.46 (br. s, 2H, $N\underline{H}_2$), 5.76 (s, 1H, $C\underline{H}$). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 28.8 ($C(\underline{C}H_3)_3$), 32.2 ($\underline{C}(CH_3)_3$), 90.6 ($\underline{C}_qCONH_2$), 99.5 ($\underline{C}H$), 154.1 ($\underline{C}_qNH_2$), 160.4 ($\underline{C}_qC(CH_3)_3$), 167.4 ($\underline{C}(O)NH_2$).

3 Biological screenings

3.1 NPP1

The inhibition of NPP1 was screened in collaboration with Prof. Müller, Pharmaceutical Institute, Pharmaceutical Chemistry I, University of Bonn, Germany. This protocol has been described in the work of Koen Muylaert.[211]

3.1.1 Materials

2-(*N*-cyclohexylamino)ethanesulfonic acid (CHES) and tris(hydroxymethyl)aminomethane (Tris) were purchased from Applichem (Darmstadt, Germany). Disodium hydrogen phosphate was obtained from Carl Roth (Karlsruhe, Germany). ATP, calcium chloride, dimethyl sulfoxide, magnesium chloride, *p*-nitrophenyl 5'-thymidine monophosphate (*p*-Nph-5'-TMP), sodium chloride and sodium hydroxide were purchased from Sigma (Steinheim, Germany). Human recombinant soluble NPP1, expressed in NS0 cells from murine myeloma, was obtained from R&D Systems GmbH (Wiesbaden, Germany). Human recombinant soluble NPP1, expressed in Sf9 insect cells, was prepared in-house.

3.1.2 First screening

The first screening of the compounds for inhibition of human NPP1 was performed using a colorimetric assay with the artificial substrate *p*-nitrophenyl 5'-thymidine monophosphate (*p*-Nph-5'-TMP).[212,213] The assays were carried out at 37 °C in a total volume of 100 µL in a clear 96-well microplate. The reaction mixture contained 1 mM $CaCl_2$, 200 µM $ZnCl_2$, 50 mM Tris, pH 9.0, 400 µM *p*-Nph-5'-TMP and 10 µM of test compound. The enzymatic reactions were initiated by the addition of 20 ng of human NPP1 (commercial, K_m = 8.17 µM), then incubated at 37 °C for 15 minutes, and subsequently terminated by the addition of 20 µL of 1.0 N NaOH. The liberated amount of *p*-nitrophenolate was measured at 400 nm. All experiments were performed two times in triplicate. The % inhibition of test compounds was determined and the blank experiment (without test compound) was set as 100% of enzyme activity.

3.2 Antibacterial activity I

The first antibacterial activity assessment was performed at the Laboratory for Microbiology (Ghent University, Belgium). A two-step procedure was proposed. In a first screening step, a large panel of compounds with unknown antimicrobial activity can be tested in a single 'high' concentration against a test panel of four bacterial test- and control strains. This screening will allow to objectively select those compounds that exhibit potential antimicrobial activity for a second series of tests in which the minimal inhibitory concentration (MIC) of the compounds can be individually determined for each compound-test strain combination.

3.2.1 Test organisms

Initially, a panel of 4 internationally used test- and control strains for antimicrobial bioassays will be used in the assays. These strains have been chosen based on the clinical relevance of the represented species as indicated in similar studies in literature. If required, additional strains can be added to the panel at later stages.

Gram-positive bacteria:

Bacillus subtilis LMG 13579 (28 °C)

Staphylococcus aureus LMG 8064 (37 °C)

Gram-negative bacteria:

Escherichia coli LMG 8063 (37 °C)

Klebsiella pneumoniae LMG 2095 (37 °C)

3.2.2 Protocol outlines

Initial screening for antimicrobial activity:

Stock solutions of test compounds are prepared in DMSO by dissolving 1-10 mg in 200 μL DMSO and adding 800 μL Mueller-Hinton (MH) culture broth. Bacterial inocula of the four test strains are prepared from overnight cultures using the growth media as specified in the BCCM/LMG strain catalogue. Inocula are prepared in MH broth until a density of 10^5 CFU/mL (or a McFarland turbidity standard equivalent) is reached. The bioassay is carried out in sterile 96-well microtiter plates. Per plate, the antimicrobial potency of max. 45 different compounds can be tested in duplicate against a single test strain. The remaining 6 wells are duplicates of positive, negative and sterility controls, respectively. To each well, 170 μL of sterile MH broth is added. Next, depending on the type of well, additional reagents are added. In each pair of test wells, 10 μL of a specific test compound stock solution is added. Test wells A1 and A2 thus contain 10 μL of compound 1, test wells A3 and A4 contain 10 μL of compound 2, etc. In the two positive control wells, 10 μL gentamicin sulfate solution (in a concentration similar to the compound concentration) is added. In the two negative control wells, 10 μL of sterile 0.85% saline is added. Finally, in all wells, 20 μL of bacterial inoculum is added. In the two sterility control wells, 20 μL sterile MH broth is added.

This way, final concentrations of 1% (v/v) DMSO, 0.05-0.5 mg/mL test compound and 10^4 CFU/mL test bacteria are obtained in a test volume of 200 μL. Depending on the test strain, plates are incubated at the respective temperatures (28 or 37 °C) for 24h under aerobic conditions.

Bacterial growth will be assessed in each well by spectrophotometric turbidity measurement at 590 nm. Turbidity levels comparable to those in the positive control wells will be regarded as positive for antimicrobial activity of the compound in question. Turbidity levels comparable to those in the negative control wells will be considered as negative for antimicrobial activity. In addition, wells will also be scored visually to potentially differentiate between bactericidal (low turbidity of supernatant without cell pellet formation) and bacteriostatic (low turbidity of supernatant with cell pellet formation) actions of the test compounds.

Determination of minimal inhibitory concentration (MIC):

The methodological setup is highly comparable to the initial screening, with the crucial difference that for MIC determinations compounds will now be tested in a range of different concentrations.

The MICs are determined by the liquid dilution method. For each of the selected test compounds exhibiting potential antimicrobial activity in the initial screening, stock solutions in a range of concentrations (*e.g.* 2.5, 5, 10, 20, 50 and 100 μg/mL) are prepared in DMSO. For comparison of MIC results, also solutions of gentamicin sulfate are prepared in the same concentration range.

For each MIC dilution series in increasing order of concentration, the first well showing lack of visible growth (i.e. exhibiting a turbidity comparable to the positive control wells) will be considered as the MIC (μg/mL) for that specific compound-test strain combination.

3.3 Antibacterial activity II

A second antibacterial activity screening was performed at the Laboratory of Pharmaceutical Microbiology (Ghent University, Belgium). This protocol has been described in the work of Elisabeth Delbeke.[214]

The antibacterial activity against *Escherichia coli* LMG 8063, *Klebsiella pneumoniae* LMG 2095, *Pseudomonas aeruginosa* PAO1, *Staphylococcus aureus* ATCC 6538 and *Staphylococcus aureus Mu50* was assessed by a broth dilution method (CLSI, Performance Standards for Antimicrobial Susceptibility Testing. Twentieth second Informational Supplement M100-S22. Wayne, PA, USA, 2012). Strains with LMG designation were obtained from the BCCM/LMG Bacteria Collection (Ghent, Belgium) while strain ATCC 6538 was obtained from the American Type Culture Collection (Manassas, VA). *Staphylococcus aureus* Mu50 was a kind gift of P. Vandamme (Ghent, Belgium). All strains were grown aerobically at 37 °C on Mueller Hinton agar (LabM, Heywood, UK). The minimal inhibitory concentration that inhibited growth by at least 50% compared to the untreated control ($MIC_{1/2}$), the minimal inhibitory concentration that inhibited growth completely (MIC) and the minimal bactericidal concentration at which no more cell viability of the test organism can be observed (MBC) were used as a measure of activity. $MIC_{1/2}$, MIC and MBC values were determined using flat-bottomed 96-well microtiter plates (TPP, Trasadingen, Switzerland). Concentrations of test compounds ranged from 0.48 to 2500 µg/mL in Mueller Hinton broth (LabM). The inoculum was standardized at approximately 5 x 10^5 colony forming units/mL. The plates were incubated at 37 °C for 24 h and the optical density was determined at 590 nm using a multilabel microtiter plate reader (Envision Xcite, PerkinElmer LAS, Waltham, MA).

3.4 Antiviral activity I

Inhibition of feline parvovirus (FPV) was performed in collaboration with Okapi Sciences, Leuven, Belgium. This protocol has been described in the work of Koen Muylaert.[211]

To CrFK cells (cat kidney cells), a 1:5 serial dilution of test compounds (50, 10, 2, 0.4 µM) and a FPV concentration (high enough to obtain a complete cytopathogenic effect in the infected cells) are added. These with FPV-infected and with test compound treated CrFK cells are incubated for three to four days at 37 °C and 5% CO_2. After this incubation period, the cytopathogenic effect is measured.

3.5 Antiviral activity II

A broad spectrum antiviral evaluation was performed in collaboration with Prof. Naesens (Rega Institute for Medical Research, KU Leuven, Belgium).

The compounds were evaluated against a broad and diverse panel of DNA- and RNA-viruses, using cell culture-based cytopathic effect (CPE) reduction and cell viability assays. The following viruses were tested on human embryonic lung (HEL) fibroblast cells: herpes simplex virus type 1 (HSV-1); a thymidine kinase-deficient (TK^-) HSV-1 strain resistant to acyclovir; herpes simplex virus type 2 (HSV-2); vaccinia virus; human adenovirus type 2 (Ad2); and human coronavirus (strain 229E). The viruses monitored on human cervix carcinoma HeLa cells were: vesicular stomatitis virus (VSV); Coxsackie B4 virus; and respiratory syncytial virus (RSV). African Green Monkey Vero cells were used to determine the antiviral effect on parainfluenza-3 virus; reovirus-1; Sindbis virus; Coxsackie B4 virus, Punta Toro virus, yellow fever virus and Zika virus. Human influenza A/H1N1, A/H3N2 and B viruses were examined on Madin-Darby canine kidney (MDCK) cells. Finally, the activity against human immunodeficiency virus type 1 and type 2 was assessed in human MT-4 lymphoblast cells.

Semiconfluent cell cultures in multiwell plates were infected with virus at a multiplicity of infection of 100 $CCID_{50}$ (50% cell culture infective dose) per well. Together with the virus, the test and reference compounds were added at serial dilutions. Two parallel plate sets were prepared: one plate set receiving compounds and virus, and one plate set receiving compounds but no virus. The plates were incubated at 37 °C (or 35 °C in the case of corona- and influenza viruses) until clear CPE was reached, i.e. during 3 to 6 days, except for Ad2 which required 10 days incubation.

In the first basic screening, the compounds' activity and cytotoxicity were measured by MTS cell viability assay. The MTS reagent (CellTiter 96® AQ_{ueous} MTS Reagent from Promega) was added, and 4 h later, absorbance at 490 nm was recorded in a plate reader. The EC_{50} (50% effective concentration) and CC_{50} (50% cytotoxic concentration) values were calculated by interpolation using semi-log dose response. The % protection against virus was defined as: $[(OD_{Cpd})virus - (OD_{Contr})virus)] / [(OD_{Contr})mock - (OD_{Contr})virus] \times 100$, where (OD_{Cpd})virus is the OD for a given concentration of the compound in virus-infected cells; (OD_{Contr})virus is the OD for the untreated virus control; and (OD_{Contr})mock is the OD for the untreated mock-infected control. The % cytotoxicity was defined as: $[1 - (OD_{Cpd})mock / [(OD_{Contr})mock] \times 100$, where (OD_{Cpd})mock is the OD for a given concentration of the compound in mock-infected wells.

Summary

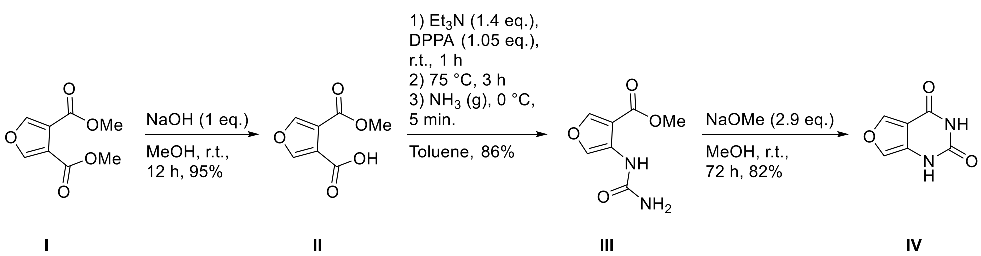
The pharmaceutical industry has already made large contributions to human healthcare and the quality of life, by providing medicines to treat a broad variety of medical conditions. However, the long list of incurable diseases and problems such as the development of resistance by pathogens against existing medicines illustrates the need for new medicines. Unfortunately, the pharmaceutical industry struggles to find new medicines for these diseases, and despite increased expenditures, the amount of new molecular entities remains relatively constant. One of the explanations for this problem is the fact that the large compound libraries, which are used in high-throughput screening, are suffering from poor chemical diversity.

To address this problem, synthetic efforts should focus on creating new scaffolds rather than changing the decoration pattern of already extensively investigated scaffolds. In addition to that, the use of smaller fragments should increase the hit rates in biological screenings. The fragment hits are expected to have lower activities compared to the hits of larger molecules, but their small size allows for modifications in order to increase the activity. Another important aspect is that the new scaffolds should be drug-like. It is useless to create new molecules which are expected to have poor pharmacokinetic properties.

In this PhD thesis, novel drug-like scaffolds were synthesized in order to increase the chemical diversity or 'chemical space'. The scaffolds were selected from the class of privileged scaffolds, which are characterized by their ability to bind to different, unrelated biological targets. This way, the newly synthesized molecules could be screened for biological activity across a broad range of therapeutic areas.

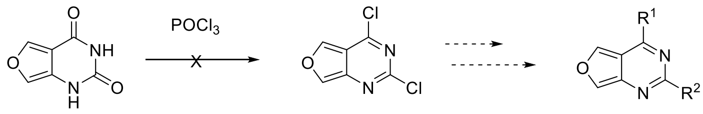
The proposed scaffolds, furo[3,4-*d*]pyrimidine and oxazolo[4,5-*d*]pyrimidine, have only been reported in the literature to a very limited extent, and a new synthetic route towards these novel, drug-like scaffolds was envisioned in this PhD research.

Starting from commercially available dimethyl furan-3,4-dicarboxylate **I**, a partial hydrolysis afforded the monocarboxylic acid **II** in excellent yield (Scheme I). This carboxylic acid was converted *via* a Curtius rearrangement reaction to the ureid **III** in very good yield. Ring closure of the ureid afforded the furo[3,4-*d*]pyrimidine-2,4-dione scaffold **IV** in good yield.



Scheme I. Three-step synthesis towards furo[3,4-d]pyrimidine-2,4-dione ***IV***

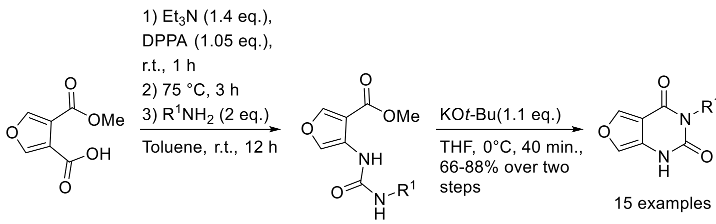
Unfortunately, all attempts to convert the furo[3,4-*d*]pyrimidine-2,4-dione scaffold **IV** to the 2,4-dichloropyrimidine **V** were unsuccessful, blocking the synthetic route towards the envisioned furo[3,4-*d*]pyrimidines **VI** (Scheme II).



IV **V** **VI**

*Scheme II. Conversion of furo[3,4-d]pyrimidine-2,4-dione **IV** to the 2,4-dichloropyrimidine **V** was not successful*

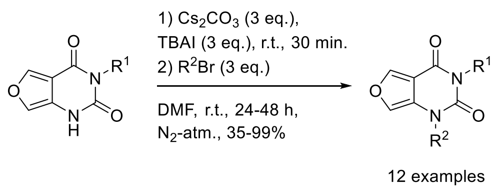
By using an appropriate amine in the Curtius rearrangement reaction, the ureids **VII** and subsequent *N*-3 functionalized furo[3,4-*d*]pyrimidine-2,4-diones **VIII** could be obtained in good yields (Scheme III).



II **VII** **VIII**

Scheme III. Straightforward synthesis of N-3 functionalized furo[3,4-d]pyrimidine-2,4-diones

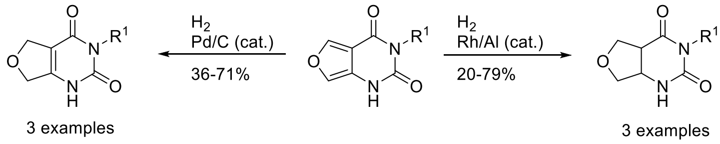
These compounds could be further functionalized by a cesium carbonate mediated alkylation of *N*(1) to afford the *N*(1)-/*N*(3)-functionalized structures **IX** (Scheme IV).



VIII **IX**

Scheme IV. Synthesis of N-1/N-3-functionalized furo[3,4-d]pyrimidine-2,4-diones

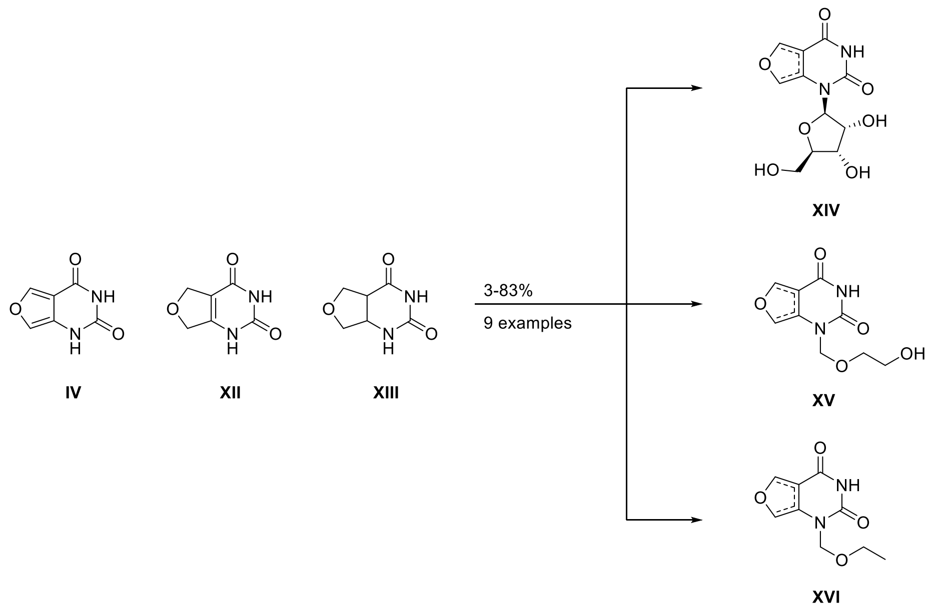
The transformation of the furan ring could be accomplished by heterogeneous catalytic hydrogenation. The use of palladium on carbon allowed the synthesis of 5,7-dihydrofuro[3,4-*d*]pyrimidine-2,4-diones **X**, while the use of rhodium on alumina afforded the tetrahydrofuro[3,4-*d*]pyrimidine-2,4-diones **XI** (Scheme V).



X **VIII** **XI**

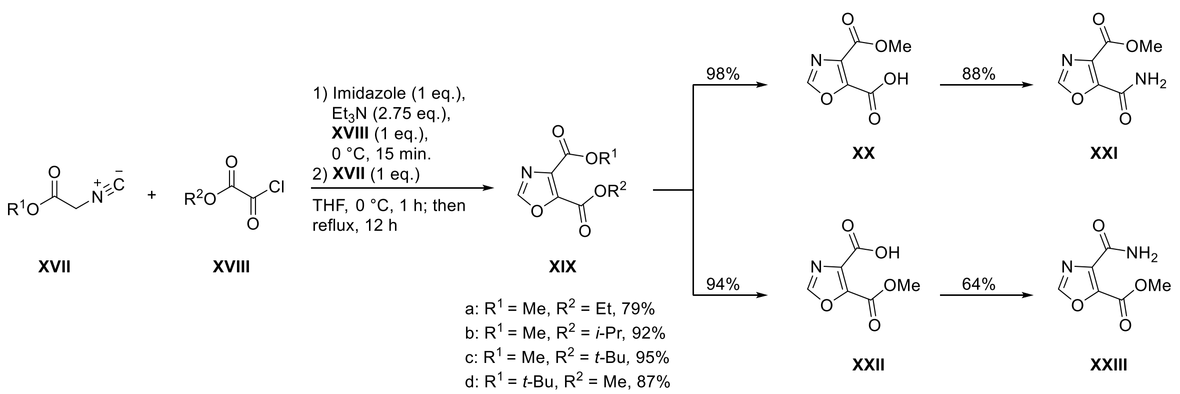
*Scheme V. Hydrogenation towards 5,7-dihydrofuro[3,4-d]pyrimidine-2,4-diones **X** and tetrahydrofuro[3,4-d]pyrimidine-2,4-diones **XI***

With the three scaffolds **IV**, **XII** and **XIII** in hand, a series of nucleoside analogues **XIV**-**XVI** was synthesized in varying yields (Scheme VI). In general, the yields of the *N*-1 functionalization reaction decreased upon increasing saturation of the furan ring.



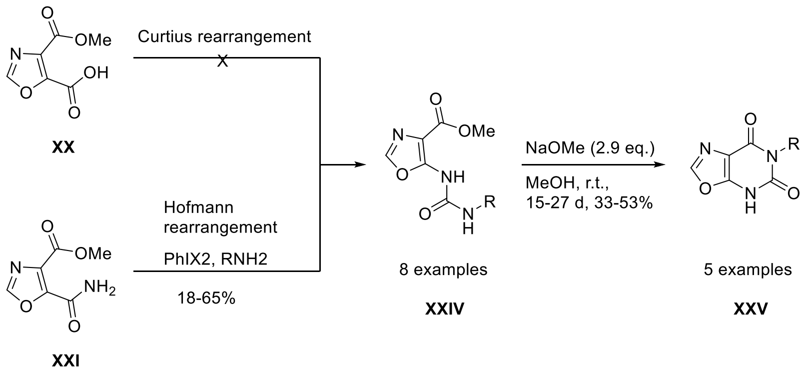
Scheme VI. Synthesis of N-1 functionalized furo[3,4-d]pyrimidine-2,4-diones

In order to gain synthetic access to the oxazolo[4,5-*d*]pyrimidine and oxazolo[5,4-*d*]pyrimidine scaffolds, the esters **XIX** were constructed in good yields by reaction of 2-chloro-2-oxoacetate **XVIII** with isocyanide **XVII** (Scheme VII). The esters **XIX** could be transformed into the carboxylic acids **XX** and **XXII** in excellent yields, by a chemoselective trifluoroacetic acid-mediated cleavage of the tert-butyl ester groups. These carboxylic acids were used as precursors for the Curtius rearrangement, and they were also further transformed into the primary carboxamides **XXI** and **XXIII**, which were used as precursors for the Hofmann rearrangement.



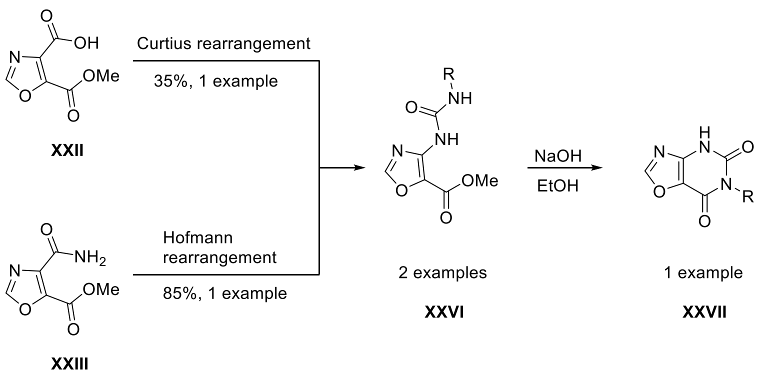
Scheme VII. Synthesis of the precursors for the Curtius and Hofmann rearrangement

The Curtius rearrangement of carboxylic acid **XX** was not successful, and a hypervalent iodine induced Hofmann rearrangement was used to convert the primary carboxamide **XXI** into the ureids **XXIV** in low to moderate yields (Scheme VIII). The use of oxidation-sensitive amines such as (activated) benzylamines proved to be problematic, limiting the scope of this reaction. The ureids were ring-closed by sodium methoxide in methanol to afford the oxazolo[5,4-*d*]pyrimidine-5,7-diones **XXV** in low to moderate yields. This ring closure was characterized by very long reaction times.



*Scheme VIII. Synthesis of the ureids **XXIV** via a Hofmann rearrangement, and ring closure towards oxazolo[5,4-d]pyrimidine-5,7-diones **XXV***

The ureids **XXVI** could be obtained via the Curtius or Hofmann reaction (Scheme IX). The Hofmann reaction resulted in a much better yield, but did not allow the use of oxidation-sensitive amines. Ring closure of ureid **XXVI** afforded oxazolo[4,5-*d*]pyrimidine-5,7-dione **XXVII** in very low yield.



*Scheme IX. Synthesis of the ureids **XXVI** via the Curtius and Hofmann rearrangement reaction, and ring closure towards oxazolo[4,5-d]pyrimidine-5,7-dione **XXVII***

The library of new molecules that resulted from this PhD research was screened for biological activity across different therapeutic areas: inhibition of NPP1, broad antibacterial activity and broad antiviral activity screening. No significant hits were detected during these biological screenings.

Samenvatting

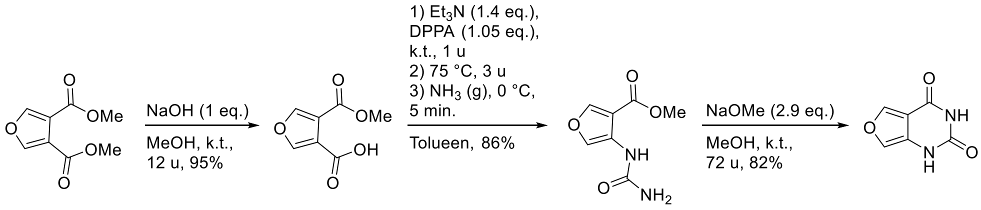
De farmaceutische industrie heeft reeds belangrijke bijdragen geleverd aan de gezondheidszorg en de levenskwaliteit, door medicijnen te ontwikkelen tegen een grote variëteit aan medische aandoeningen. Er zijn echter nog heel wat ongeneeslijke ziekten waarvoor geen effectief geneesmiddel ter beschikking is, en er zijn ook problemen zoals de ontwikkeling van resistentie door pathogenen tegen de huidige medicijnen. Dit illustreert de nood aan de ontwikkeling van nieuwe geneesmiddelen. De farmaceutische industrie heeft echter moeilijkheden om nieuwe medicijnen tegen deze aandoeningen te ontwikkelen, en ondanks een toename in de uitgaven voor onderzoek en ontwikkeling, resulteert dit niet in een toename van het aantal goedgekeurde nieuwe medicijnen. Een van de oorzaken van dit probleem is de lage chemische diversiteit in de molecuulbibliotheken die gebruikt worden in de zoektocht naar nieuwe actieve stoffen.

Om dit probleem aan te pakken moet er gefocust worden op de synthese van nieuwe basisstructuren, in plaats van oppervlakkige wijzigingen aan te brengen aan basisstructuren die reeds uitvoerig onderzocht zijn. Daarbovenop zullen er ook kleinere fragmenten aangemaakt worden, omdat dit meer kans geeft op het ontdekken van biologische activiteit. De activiteit zal naar verwachting wel minder sterk zijn dan voor grotere moleculen, maar door hun lage molecuulmassa is er nog mogelijkheid om structurele aanpassingen te maken om zo de activiteit te verhogen. Een ander belangrijk aspect is dat deze nieuwe basisstructuren medicinaal interessant moeten zijn. Het heeft geen zin om nieuwe moleculen te maken als er verwacht wordt dat deze slechte farmacokinetische eigenschappen zullen bezitten.

In dit doctoraatsonderzoek werden nieuwe, medicinaal interessante basisstructuren ontwikkeld om zo de chemische diversiteit te vergroten en onbekende ‘chemische ruimte’ te betreden. De basisstructuren werden geselecteerd uit de klasse van de geprivilegieerde structuren, welke gekenmerkt worden door hun activiteit tegenover diverse, niet aan elkaar verwante biologische doelwitten. Op deze manier zouden de nieuwe structuren onderzocht kunnen worden op biologische activiteit in diverse therapeutische domeinen.

De vooropgestelde basisstructuren, furo[3,4-*d*]pyrimidine en oxazolo[4,5-*d*]pyrimidine, zijn slechts zeer schaars onderzocht geweest op zowel synthetisch als biologisch vlak, en een nieuwe syntheseroute om deze medicinaal interessante structuren aan te maken en te kunnen onderzoeken werd dan ook beoogd in dit doctoraatsonderzoek.

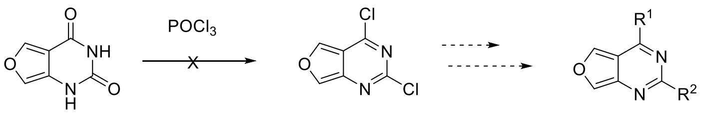
Uitgaande van het commercieel beschikbare dimethyl furan-3,4-dicarboxylaat **I** werd een partiële hydrolyse uitgevoerd naar het enkelvoudig carbonzuur **II** (Schema I). Dit carbonzuur werd verder omgezet via een Curtius omleggingsreactie tot het ureïde **III**, dat met een heel goed rendement bekomen werd. De daaropvolgende ringsluiting van het ureïde leverde de furo[3,4-*d*]pyrimidine-2,4-dion basisstructuur **IV** op met een goed rendement.



I II III IV

Schema I. Synthese in drie stappen naar furo[3,4-d]pyrimidine-2,4-dion ***IV***

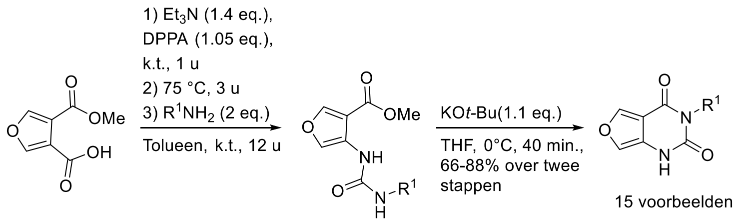
Jammer genoeg mislukten alle pogingen om het furo[3,4-*d*]pyrimidine-2,4-dion **IV** verder om te zetten naar het 2,4-dichloorpyrimidine **V** (Schema II). Hierdoor was de syntheseroute naar de beoogde furo[3,4-*d*]pyrimidines **VI** geblokkeerd.



IV V VI

Schema II. De omzetting van furo[3,4-d]pyrimidine-2,4-dion ***IV*** *naar het 2,4-dichloorpyrimidine* ***V*** *was niet succesvol*

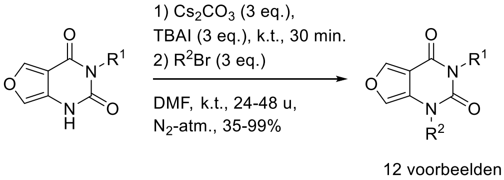
Door een geschikt amine te gebruiken in de Curtius omleggingsreactie konden de ureïden **VII** en de daar uit voortvloeiende *N*-3 gefunctionaliseerde furo[3,4-*d*]pyrimidine-2,4-dionen **VIII** bekomen worden met goede rendementen (Schema III).



II VII VIII

Schema III. De efficiënte synthese van N-3 gefunctionaliseerde furo[3,4-d]pyrimidine-2,4-dionen

Deze moleculen werden verder gefunctionaliseerd door een alkylering uit te voeren op het *N*-1 stikstofatoom. Deze transformatie werd bewerkstelligd door het gebruik van cesium carbonaat, en leverde de *N*-1/*N*-3-gefunctionaliseerde structuren **IX** op (Schema IV).

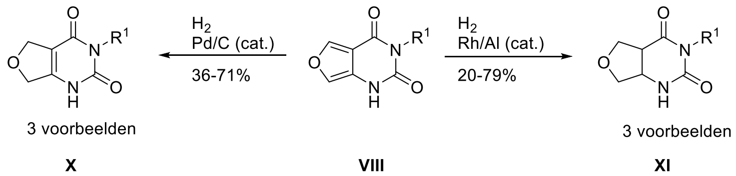


VIII IX

Schema IV. De synthese van N-1/N-3-gefunctionaliseerde furo[3,4-d]pyrimidine-2,4-dionen

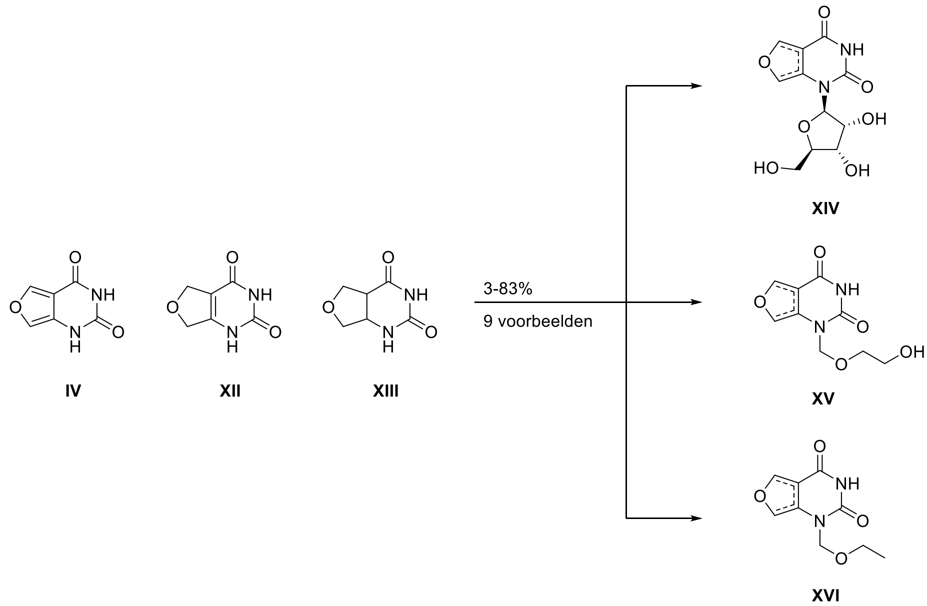
De furan ring kon getransformeerd worden door middel van een heterogene katalytische hydrogenatie. Het gebruik van palladium op koolstof gaf aanleiding tot de vorming van de 5,7-

dihydrofuro[3,4-*d*]pyrimidine-2,4-dionen **X**, terwijl het gebruik van rhodium op alumina de tetrahydrofuro[3,4-*d*]pyrimidine-2,4-dionen **XI** opleverde (Schema V).



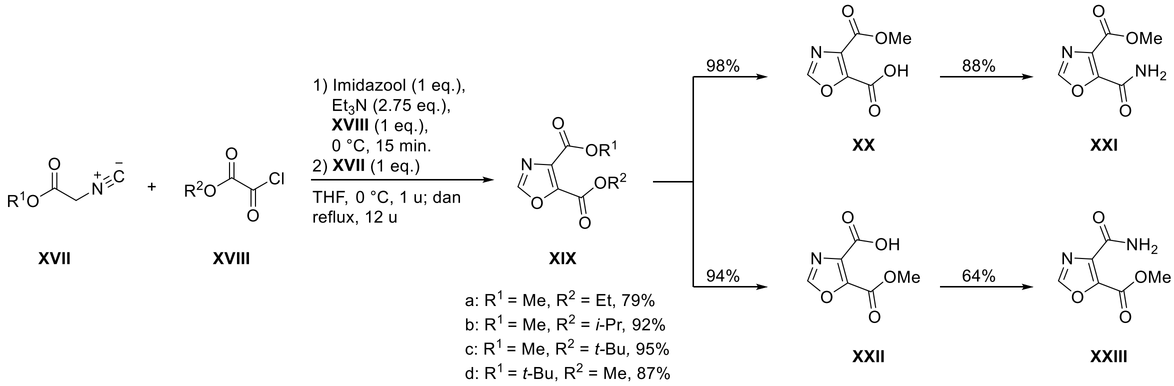
*Schema V. De synthese van 5,7-dihydrofuro[3,4-d]pyrimidine-2,4-dionen **X** en tetrahydrofuro[3,4-d]pyrimidine-2,4-dionen **XI***

Met de basisstructuren **IV**, **XII** en **XIII** ter beschikking kon een serie van nucleoside analogen **XIV-XVI** aangemaakt worden (Schema VI). Het rendement van deze reacties vertoonde een algemene dalende trend naarmate de verzadiging van de furan ring toenam.



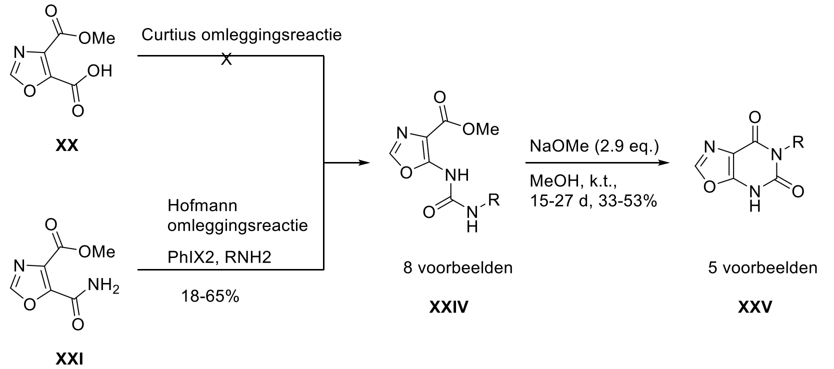
Schema VI. De synthese van N-1 gefunctionaliseerde furo[3,4-d]pyrimidine-2,4-dionen

Om toegang te krijgen tot de oxazolo[4,5-*d*]pyrimidine en oxazolo[5,4-*d*]pyrimidine basisstructuren, werden de esters **XIX** aangemaakt met goede rendementen door een reactie tussen het 2-chloor-2-oxoacetaat **XVIII** en het isocyanide **XVII** te bewerkstelligen (Schema VII). Deze esters konden verder getransformeerd worden naar de carbonzuren **XX** en **XXII** met uitstekende rendementen, dankzij een chemoselectieve afsplitsing van de tertiair-butyl ester groep door middel van trifluorazijnzuur. Deze carbonzuren werden gebruikt in de Curtius omleggingsreactie, en deze werden ook verder omgezet naar de primaire amiden **XXI** en **XXIII**, welke gebruikt werden in de Hofmann omleggingsreactie.



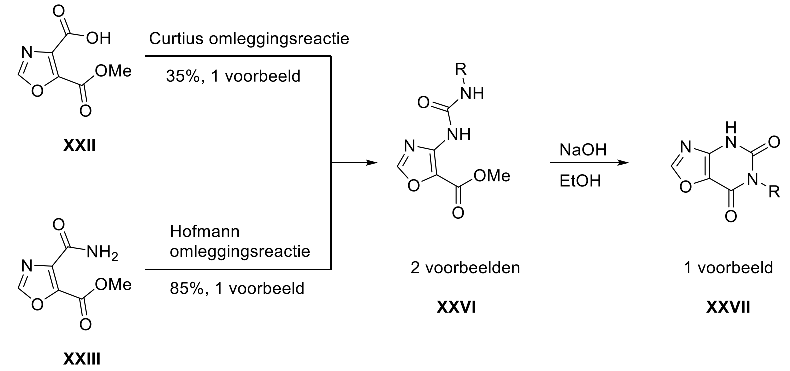
Schema VII. De synthese van de precursoren voor de Curtius en Hofmann omleggingsreactie

Aangezien de Curtius omleggingsreactie niet bleek te werken met het carbonzuur **XX**, werd een Hofmann omleggingsreactie uitgevoerd op het primaire amide **XXI** om zo de ureïden **XXIV** te bekomen met lage tot matige rendementen (Schema VIII). Deze Hofmann omleggingsreactie werd bewerkstelligd door het gebruik van een hypervalent jood-reagens. Het gebruik van amines die gevoelig zijn voor oxidatie bleek echter problematisch te zijn, wat het toepassingsbereik van deze reactie inperkte. De ureïden werden ringgesloten door middel van natriummethoxide in methanol tot de oxazolo[5,4-*d*]pyrimidine-5,7-dionen **XXV** die met lage tot matige rendementen bekomen werden. Deze ringsluitingsreactie werd gekenmerkt door erg lange reactietijden.



*Schema VIII. De synthese van de ureïden **XXIV** via een Hofmann omleggingsreactie, en ringsluiting tot de oxazolo[5,4-d]pyrimidine-5,7-dionen **XXV***

De ureïden **XXVI** konden bekomen worden door zowel een Curtius als een Hofmann omleggingsreactie (Schema IX). De Hofmann reactie gaf wel een beter rendement, maar liet het gebruik van oxidatiegevoelige amines niet toe. Ringsluiting van ureïde **XXVI** leverde oxazolo[4,5-*d*]pyrimidine-5,7-dion **XXVII** op met een zeer laag rendement.



Schema IX. De synthese van de ureïden ***XXVI*** *via de Curtius en Hofmann omleggingsreactie, en de ringsluitingsreactie naar oxazolo[4,5-d]pyrimidine-5,7-dion* ***XXVII***

De bibliotheek aan nieuwe moleculen die in dit doctoraatsonderzoek werd gesynthetiseerd, werd onderzocht op biologische activiteit in verschillende therapeutische domeinen: inhibitie van NPP1, brede antibacteriële activiteit en brede antivirale activiteit. Er werden echter geen significante activiteiten waargenomen tijdens deze biologische onderzoeken.

Curriculum Vitae

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